CITY OF INDIANAPOLIS

WILLIAM H. HUDNUT, III
MAYOR

Director
Richard Rippel

DEPARTMENT OF PUBLIC WORKS
2460 CITY-COUNTY BUILDING
INDIANAPOLIS, INDIANA 46204

July 15, 1982

Mr. Robert Robichaud
Pretreatment Program Coordinator
Permit Branch
U.S. Environmental Protection Agency
Region V
230 South Dearborn Street
Chicago, IL 60604

RE: Indianapolis Pretreatment Program
Pilot Plant Plan of Operation

Dear Bob:

Please find enclosed the Plan of Operation for the Pretreatment Pilot Plant of the City of Indianapolis.

I am looking forward to seeing you during your visit to Indianapolis in the last week of July and discuss with you at that time the City's Pretreatment Program.

If you have any questions, please call me.

Sincerely,

V. Keramida

Vicky Keramida, Ph.D.
Project Manager
Industrial Pretreatment Program

VK/nlt

cc: Susan Loudermilk
Pat Stevens
File, 1A.1, Industrial Pretreatment

CITY OF INDIANAPOLIS
DEPARTMENT OF PUBLIC WORKS

INDUSTRIAL PRETREATMENT PROGRAM

FINAL PILOT PLANT PLAN OF OPERATION

Peat, Marwick, Mitchell & Co.

JAMES M. MONTGOMERY
CONSULTING ENGINEERS, INC.

EMS Laboratories/
Mark Battle Associates, Inc.

APRIL 1982

CITY OF INDIANAPOLIS

WILLIAM H. HUDNUT, III
MAYOR

Director
Richard Rippel

DEPARTMENT OF PUBLIC WORKS
2460 CITY-COUNTY BUILDING
INDIANAPOLIS, INDIANA 46204

July 15, 1982

Mr. Robert Robichaud
Pretreatment Program Coordinator
Permit Branch
U.S. Environmental Protection Agency
Region V
230 South Dearborn Street
Chicago, IL 60604

RE: Indianapolis Pretreatment Program
Pilot Plant Plan of Operation

Dear Bob:

Please find enclosed the Plan of Operation for the Pretreatment Pilot Plant of the City of Indianapolis.

I am looking forward to seeing you during your visit to Indianapolis in the last week of July and discuss with you at that time the City's Pretreatment Program.

If you have any questions, please call me.

Sincerely,

V. Keramida

Vicky Keramida, Ph.D.
Project Manager
Industrial Pretreatment Program

VK/nlt

cc: Susan Loudermilk
Pat Stevens
File, 1A.1, Industrial Pretreatment

CITY OF INDIANAPOLIS
DEPARTMENT OF PUBLIC WORKS

INDUSTRIAL PRETREATMENT PROGRAM

FINAL PILOT PLANT PLAN OF OPERATION

Peat, Marwick, Mitchell & Co.

JAMES M. MONTGOMERY
CONSULTING ENGINEERS, INC.

EMS Laboratories/
Mark Battle Associates, Inc.

APRIL 1982

JAMES M. MONTGOMERY CONSULTING ENGINEERS, INC.

2255 Ygnacio Valley Road, Suite C, Walnut Creek, California 94598 / (415) 933-2250

April 28, 1982

Mr. Richard Rippel
Director, Department of Public Works
City of Indianapolis
2460 City-County Building
Indianapolis, IN 46204

Subject: Indianapolis Pretreatement Program
Pilot Plant Plan of Operation

File No. 1180.0010

Dear Mr. Rippel:

Please find enclosed the final Plan of Operations for the pretreatment pilot plant. This document, which was first issued in draft form on September 15, 1981, has gone through a number of revisions as comments from EPA were incorporated, and as the detailed planning for the pilot plant was completed. The enclosed version will be the last and final one, as we are now well into the actual operation of the pilot plant. Any deviations from the program detailed in the Plan of Operation will be specifically called out in our Pilot Plant Report, which is due to be issued in draft form in August of 1982.

In accordance with a request from EPA, please forward three copies of the Plan of Operation to Mr. Bramscher of EPA, Region V, to the attention of Mr. N. Damato and Mr. R. Robichaud (2 copies). We have provided the required extra copies in the enclosure to Ms. Loudermilk.

If you have any questions, please call me.

Very truly yours,

C. B. Cain

Christopher B. Cain

/lmr

Enclosure

cc: J.S. Loudermilk (w/6 enclosure copies), Indianapolis DPW
A. McFearin, Indianapolis DPW
D. Pool, Indianapolis Belmont WTP
M. Robson, Indianapolis Belmont WTP
D. Wells, Indianapolis Belmont WTP
R. Watt, Indianapolis Belmont WTP
D. Bertelson, Indianapolis Belmont WTP
W. Bernhardt, Indianapolis Belmont WTP
S. Gohmann, EMS Laboratories
R. Wukasch, Purdue University
L. Scully, PMM (3 copies)

PLAN OF OPERATION

INDIANAPOLIS PRETREATMENT PROGRAM

PILOT PLANT

APRIL 1981

PLAN OF OPERATION
INDIANAPOLIS PRETREATMENT
PILOT PLANT

TABLE OF CONTENTS

I INTRODUCTION

The Department of Public Works of the City of Indianapolis, Indiana, is performing an Industrial Pretreatment Study in accordance with the EPA and State of Indiana guidelines. The fundamental goal of the Indianapolis Pretreatment Program is the control of industrial waste discharges to City sewers. The primary control tool will be the new Industrial Waste Ordinance.

The new Ordinance will be developed from the existing Industrial Waste Ordinance, and will be updated to meet EPA and State Pretreatment Regulations. The numerical standards used in the ordinance will be based upon the technical information developed during the City's pretreatment program.

A pilot plant, simulating the performance of the two new AWT plants which are being constructed at Indianapolis, is required in order to evaluate the impact of industrial waste and Priority Pollutants on future wastewater treatment. The AWT plants will employ primary clarifiers, trickling filters, pure oxygen activated sludge treatment, dual media filters, and ozonation for disinfection. The construction and operation of the pretreatment pilot plant (Task 4(A)) will identify any industrial wastes, particularly Priority Pollutants, which may interfere with the operation of the two AWT's or which may pass through inadequately treated and potentially impact the White River. Task 4(A) will thus technically substantiate the levels of industrial waste discharge to be permitted under the new Industrial Waste Ordinance.

This plan of operation is intended to define and schedule the activities that will take place during the operation of the pilot plant.

II OBJECTIVES

General:

A. Provide technical justification for pretreatment standards in the Industrial Waste Ordinance.

B. Aid in the optimization of the operation of the Advanced Wastewater Treatment (AWT) facilities, to maximize Priority Pollutant control.

Specific:

1. Evaluate removal of Priority Pollutants in the existing POTW's.

2. Evaluate the tolerance of the existing POTW's for Priority Pollutants.

3. Evaluate removal of Priority Pollutants in the AWT.

4. Evaluate tolerance of AWT for Priority Pollutants.

5. Evaluate the condition of the White River in terms of Priority Pollutants from the existing POTW's, as indicated by toxicity and accumulation in the benthos. Make predictions of the condition of the river after the AWT's start operations.

6. Evaluate Priority Pollutant removal enchancement through operational changes.

7. Evaluate the feasibility of Priority Pollutant removal enhancement through possible design modifications.

8. Establish the numerical pollutant limits required in the Industrial Waste Ordinance to protect the AWT plants.

III BACKGROUND

The City of Indianapolis is currently constructing two large Advanced Waste Treatment facilities to treat the wastewater produced by the citizens and industry located within Marion County.

At the Belmont treatment facility, a significant portion of the wastewater is discharged by industry, while at the Southport treatment facility the wastewater comes from the municipal sector. There are several large industries located in the Marion County area, including numerous pharmaceutical plants, automotive assembly plants, and other heavy industries. The potential for Priority Pollutant discharge is significant, and the need for a pretreatment program is substantial.

Due to the large number of industries, it is important that the industrial waste ordinance be properly documented to minimize the potential duplication of City treatment by the installation of pretreatment facilities in industrial plants located in Indianapolis. The industries in Indianapolis accept that they must discharge wastewater that is suitable for treatment in the Advanced Waste Treatment facilities. However, they are also concerned about the potential cost impact that the installation of pretreatment facilities would have on their operations costs. As a result, substantial efforts are being made to provide the technical information required to justify the establishment of discharge concentration limits on various pollutants. The establishment of the categorical pretreatment standards by EPA fo the various industries located in Indianapolis lagging behind due to the technical complications, and it is important to establish a reasonable and enforceable ordinance as soon as possible to protect the operation of the $300,000,000 treatment facilities currently under construction.

Additional concerns in the Indianapolis area involve the discharge of approximately 250 mgd of treated wastewater into the White River where the ten-year, seven-day flow is only about 35 mgd. Obviously, the impact of these discharges is substantial on this reach of the White River. It is important that the work required to characterize this impact be done as soon as possible, to take advantage of the opportunity to establish baseline data to evaluate the improvement in Priority Pollutant removal on the White River system attributable to the AWT plants. Currently, there is limited data available on the accumulation of Priority Pollutants in the water column organisms or the benthos. It is necessary to collect information on these organisms to establish a baseline and to evaluate the need for industrial pretreatment or additional treatment at the Indianapolis Wastewater Treatment Facilities.

The White River has the potential for being an important recreational area, and the evaluation of Priority Pollutant removal and/or control is an important factor to both downstream users of the water and to those desiring to use the river for recreational purposes. The impact of these discharges may be significant and is deserving of evaluation in the interest of public health.

The purpose of the proposed study is to establish a technically sound Industrial Waste ordinance for discharges to the Indianapolis wastewater system. The primary emphasis is on establishing meaningful Priority Pollutant concentration limits to protect the AWT facilities currently under construction. Substantial funds have been committed to both the planning, and to the construction of these facilities. Ongoing efforts are being expended for the control of combined sewer overflows, and for the study of sludge management alternatives. Both of these studies interact with the Industrial Pretreatment Program due to the impact of Priority Pollutant accumulation and/or discharge via these routes. Priority Pollutants which enter the treatment facility are either discharged in the effluent, accumulated in the sludge, or degraded during treatment. As a

result, the impact of inadequate control of these materials may result in substantial impact on the White River system or on the economics and feasibility of sludge disposal.

IV SUMMARY TASK LIST

		Addresses Objective
4-A.1	Review and summarize existing POTW information (Includes 4-A.2.1)	1,2
4-A.2.2	Collect and analyze samples from the POTW's during upsets.	1,2
4-A.2.3	Establish operator reporting system	1,2
4-A.3.1	Conduct mass balance around POTW's	1,2
4-A.3.2	Conduct bioassay studies on POTW's	1,5
4-A.4.3	Construct pilot plant (Includes 4-1.4.1 and 4-A.4.2)	3,4
4-A.4.4.1	Tolerance testing: Part 1: Design conditions Part 2: Spiking	3,4
4-A.4.4.2	Removal evaluation Part 1: Design conditions Part 2: Spiking	3,4

4-A.4.4.3	Analyze off gases	3
4-A.4.4.4	Run bioassays on pilot effluent	3,5
4-A.4.4.5	Test operational modifications:	6
	Part 1: Ozone dose	
	Part 2: Other	
4-A.4.4.6	Test design modifications:	7
	Part 1: Off-line	
	Part 2: On-line	
4-A.4.4.7	Prepare pilot plant report	A
4-A.5.1	Review data regarding White River Water Quality (Includes 4-A.5.2, 4-A.5.3, 4-A.5.4)	5
4-A.5.5	Analyze fish tissue samples (Deferred)	5
4-A.5.6	Conduct bioassays on peak sewer flows (Deferred)	5
4-A.5.7	Analyze river sediment	5
4-A.5.10	Prepare water quality report (Includes 4-A.5.8, 4-A.5.9)	
4-A.6	Prepare sewer use ordinance support document	

(Includes 4-A.6.1 through 4-A.6.4)

V SCHEDULE

The schedule for the construction and operation of the pilot plant, and for the related activities in Task 4 is presented by means of the following charts and tables. Table V-1, Task 4 schedule presents the overall schedule for all task 4 activities. Table V-2, Summary Pilot Plant Experimental Schedule, and Table V-3, Detailed Pilot Plant Operating Schedule, show the operating schedule for the Pilot Plant in terms of process control variables. Table V-3 tells what flows and loads will be input to which units during each month. Both tables also list when operational monitoring sampling and analytical work will be taking place, and when sampling for record will take place. Operational monitoring tests are those required to keep the pilot plant operating and are limited to conventional pollutant analyses run on-site. Sampling for record includes the metals, GC/ECD, and GC/MS analytical work needed to determine the presence and behavior of priority pollutants in the pilot plant. Table V-4 is a copy of the draft Operational Monitoring Data Sheet, showing the measurements and analytical results to be recorded each day. Table V-5 shows the schedule of record sampling to be performed during a typical month of pilot plant operation. See section VII for explanation of the Test Batteries.

VI DISCUSSION OF TASKS, INCLUDING SAMPLING AND ANALYTICAL WORK

4(A)1 EXISTING POTW DATA REVIEW

Review and summarize existing POTW information, including flow records, organic loading records, solids loading records, and sludge evaluation study data collected over the last three years. Interview City operators. Analyze statistical variability of collected data. Prepare a summary report on operations at the Belmont and Southport plants. (Interface with Task 3). (Includes Task 4(A)2.1).

TABLE V-1

CITY OF INDIANAPOLIS
PRETREATMENT PROGRAM
MONTGOMERY ENGINEERS

TASK 4 SCHEDULE

SCHEDULE REVISION DATE: MAR 5, 1982

PROJECT 1180.0010 V-1
PILOT PLANT PLAN OF OPERATION
C.B.CAIN

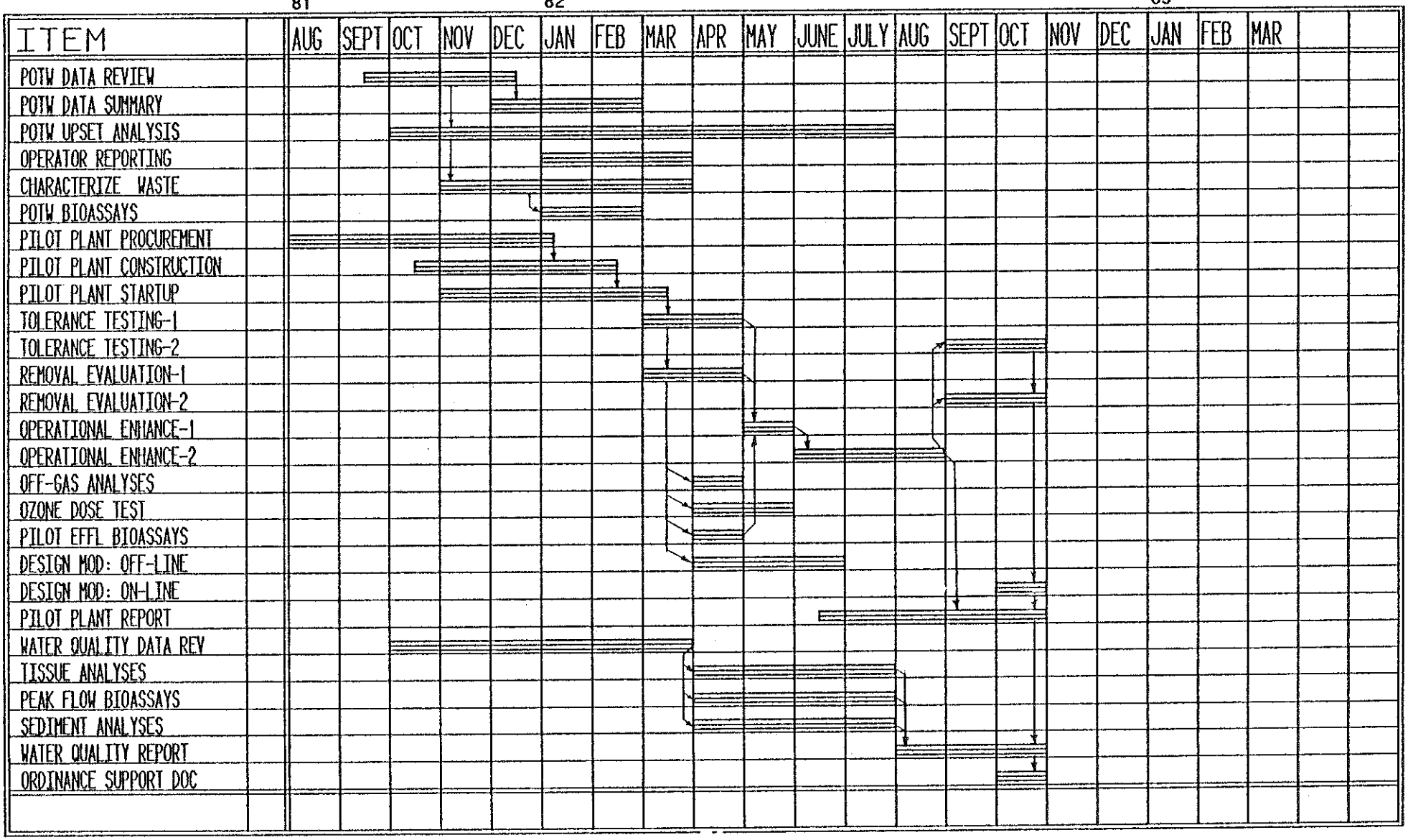


TABLE V-2

SIMPLIFIED PILOT PLANT EXPERIMENTAL SCHEDULE

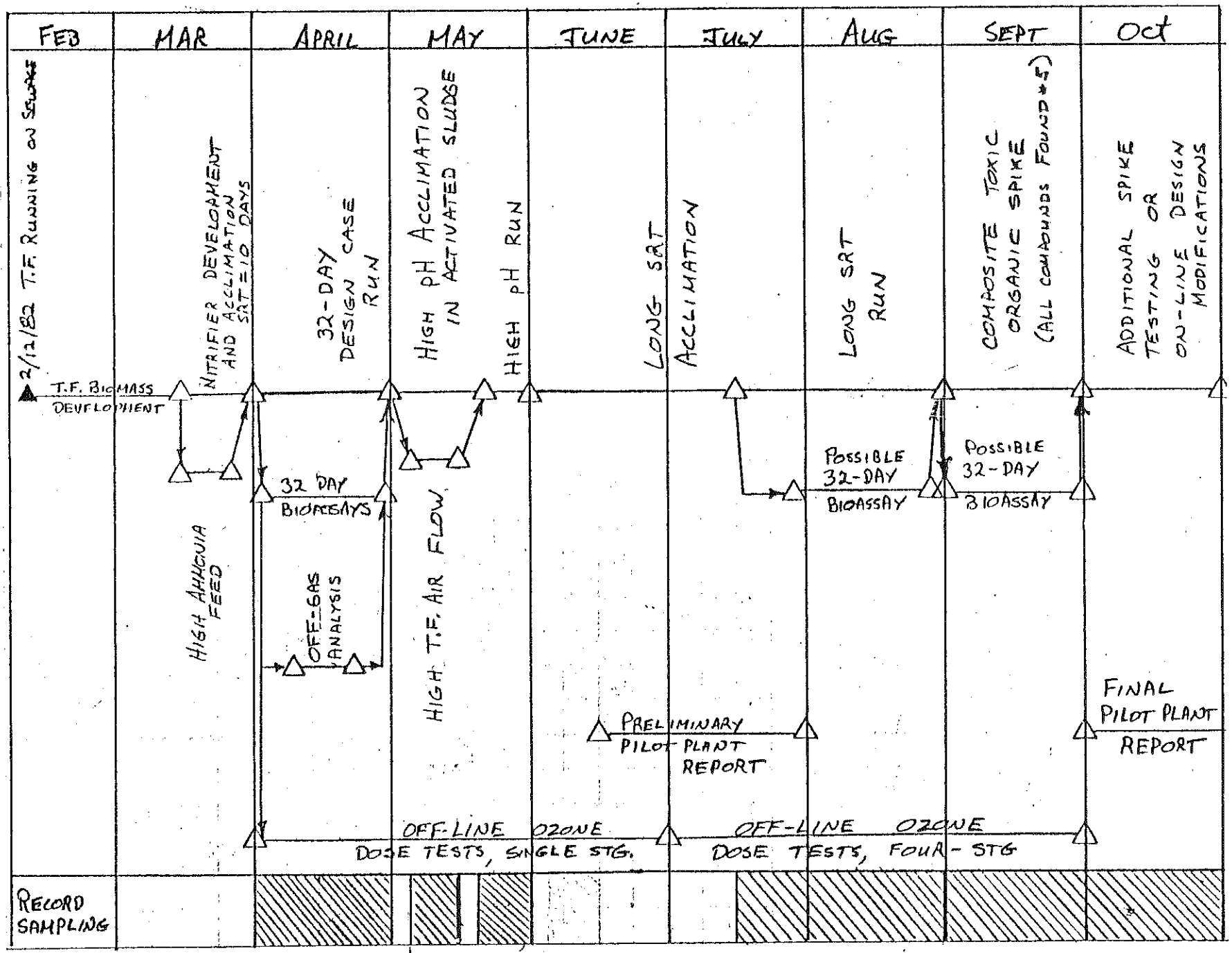


TABLE V 3

DETAILED PILOT PLANT OPERATING CRITERIA SCHEDULE
INDIANAPOLIS PRETREATMENT PROGRAM

	MONTH NUMBER:	1	2	3	4	5	6	7	8	9	10	11
	MONTH:	DEC	JAN	FEB	MAR	APRIL	MAY	JUNE	JULY	AUG	SEPT	OCT
o	MODE OF OPERATION	START-UP		ACCLIMATE		DESIGN CASE	OPERATIONAL ENHANCE-1	ACCLIMATE		OPERATIONAL ENHANCE-2	SPIKES	DESIGN MOD
	__ Source of Influent					BELMONT PRIMARY EFFLUENT						
	__ Compounds Added	NO COMPOUNDS ADDED			NH_3	NO ADDITIONS	NaOH $Ca(OH)_2$		NO ADDITIONS		PRIORITY POLLUTANT MIXTURE	
	__ Sampling Schedule	WASTE CHARACTERIZATION SAMPLING IN POTW		OPERATIONAL MONITORING		RECORD SAMPLING		OPERATIONAL MONITORING		RECORD SAMPLING		
o	TRICKLING FILTER						1.25 GPM/FT^2					
	__ Loading Rate (GPM/Ft^2)											
	__ Flow Rate (GPM)						9 GPM					
	__ Air Flow			NATURAL DRAFT 1-10 SCFM			FORCED AIR 100-1000 SCFM			NATURAL DRAFT 1-10 SCFM		
o	PURE O_2 ACTIVATED SLUDGE						7.2 GPM					
	__ Flow Rate (GPM)											
	__ Hydraulic Retention Time (Hours)						3.7 HOURS					
	__ F/M_2 MLSS			F/M=0.15,	MLSS=5000			MLSS INCREASES TO MAX CLAR. CAPACITY, F/M DECREASES			MLSS = 5000 F/M = 0.15	
	__ Solids Retention Time (Days)			10 to 13	DAYS			SRT INCREASE TO EQUILIB LEVEL = 20 DAYS			10-13 DAYS	
	__ Aeration pH			NORMAL, PH X	6.5		HIGH PH X 8.0			NORMAL, PH 6.5		
o	DUAL MEDIA FILTERS							0.86 GPM EACH, 1.72 GPM TOTAL				
	__ Flow Rate (GPM)											
	__ Loading Rate (GPM/FT^2)							3.89 GPM/FT^2				
o	OZONE DISINFECTION: UNIT 1						5.1 mg/l				VARY PER TABLE 5.1	
	__ Ozone Dose											
	__ Ozone Concentration						2%				VARY PER 5.1	
	__ Flow Rate							0.86 GPM				
	__ HRT							15 MINUTES IN 4 STAGES				
o	OZONE DISINFECTION: UNIT 2					VARY PER TABLE 5.1				5.1 mg/l		
	__ Ozone Dose											
	__ Ozone Concentration					VARY PER 5.1				2%		
	__ Flow Rate							0.86 GPM				
	__ HRT							15 MINUTES IN ONE STAGE				
o	CONTINUOUS BIOASSAY UNIT		EXISTING POTW INFLUENT			O_3 UNIT 1 EFFLUENT		PEAK SEWER FLOWS		O_3 UNIT 2 EFFLUENT		
	__ Feed Source											

Table V-4

OPERATIONAL MONITORING DATA SHEET

INDIANAPOLIS PRETREATMENT PILOT PLANT

SHEET 1 OF 2 DRAFT 11/81

DATE:	MON	TUES	WED	THUR	FRI	SAT	SUN
T.F. PUMP GPM							
CLAR. EFFL GPM							
RAS PUMP GPM							
WAS PUMP GPM							
WAS PUMP TIME ON							
FILTER 1 GPM							
FILTER 2 GPM							
VENT GAS O_2 %							
LEL %							
MIXER 1 WATTS							
MIXER 2 WATTS							
MIXER 3 WATTS							
MIXER 4 WATTS							
GAS RECIRC-1 SCFM							
GAS RECIRC-2 SCFM							
GAS RECIRC-3 SCFM							
GAS RECIRC-4 SCFM							
STAGE 1 D.O.							
STAGE 2 D.O.							
STAGE 3 D.O.							
STAGE 4 D.O.							
STAGE 1 pH							
STAGE 2 pH							
STAGE 3 pH							
STAGE 4 pH							
STAGE 1 TEMP °C							
STAGE 4 TEMP °C							
O_2 SUPPLY SCF/DAY							
SHIFT 1 : 30 MIN. SL. VOL							
SHIFT 2 : 30 MIN. SL. VOL							
SHIFT 3 : 30 MIN. SL. VOL							
T.F. INFL COD							
AER. INFL COD							
CLAR EFFL COD							
FILTER EFFL COD							
O_3 EFFL COD							
AER. INFL SS							
CLAR. EFFL SS							
RAS SS							
FILTER EFFL SS							
MLSS / MLVSS							
VENT GAS O_3 %							
F/M							
SRT							

OPERATIONAL MONITORING DATA SHEET

INDIANAPOLIS PRETREATMENT PILOT PLANT

SHEET 2 OF 2 DRAFT 11/81

	MON	TUES	WED	THUR	FRI	SAT	SUN
DATE:							
BOD: T.F. INFL							
AER. INFL							
CLAR. EFFL							
FILTER EFFL							
OZONE EFFL							
NH_3-N: AER INFL							
CLAR. EFFL.							
NO_3-N: AER. INFL							
CLAR. EFFL.							
NO_2-N: CLAR EFFL							
T.F. Infl. pH							
CLAR Effl. pH							
Aer Infl. pH							
T.F. Infl Temp °C							
Aer Infl. Temp °C							

TABLE V-5

MONTHLY RECORD SAMPLING SCHEDULE

INDIANAPOLIS PRETREATMENT PILOT PLANT

X INDICATES SAMPLE TO BE ANALYZED

DRAFT 11/81

SAMPLE POINT NO.	DATE:	MO	WE	FR	MO	WE	FR	MO	WE	FR	MO	WE	FR	MO	WE
1	T.F. INFL:	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	- BATTERY B		X			X			X			X			X
	- BATTERY D		X						X			X			
	- GC/ECD					X									
	- GC/MS														
2	AER. INFL:	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	- BATTERY B		X			X			X			X			X
	- BATTERY D		X			X			X			X			
	- GC/ECD														
3	CLAR EFFL:	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	- BATTERY B		X			X			X			X			X
	- BATTERY D		X			X			X			X			
	- GC/ECD														
* 4	FILTER EFFL:	X	X	X	X	X	X	X	X	X	X	X	X	X	X
5	- BATTERY B					X			X						
	- BATTERY D					X			X						
	- GC/ECD														
6	OZONE EFFL: 1	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	- BATTERY B		X			X			X			X			X
	- BATTERY D		X						X			X			
	- GC/ECD					X									
	- GC/MS														
10	W.A.S.:				X			X			X				
	- BATTERY C							X							
	- GC/ECD														
11	PRIM. SLUDGE:				X			X			X				
	- BATTERY C							X							
	- GC/ECD														
12	PRIM INFL:					X									
	- BATTERY B					X									
	- BATTERY D					X									
	- GC/MS														
** 7	OZONE EFFL: 2	X		X		X			X			X			X
	- BATTERY A														

4(A)2.2 EXISTING POTW UPSET ANALYSES

Collect and analyze influent samples from the existing POTW's during upsets, whenever they occur during the study period. Designation of upset events will be based on SVI, effluent SS, visual observations, and judgements by the City's operation personnel and JMM pilot plant engineers.

The purpose of Task 4(A)2.2 is to identify constituents in the wastewater which may have caused or contributed to the treatment plant upset. Identification of such constituents would permit further characterization of their impact on the AWT system during the tolerance testing phase of the pilot plant study. The information obtained during this task would also be used in the development of the sewer use ordinance discharge parameters.

The basic analytical tests for this task are given in Test Battery A in Table VII-1. Further analyses would be performed with the GC/MS to provide an in-depth analysis of the wastewater should the basic analytical tests be inconclusive. For example, if the GC/ECD scan shows interesting unknown peaks, GC/MS will be run to identify them. The samples will be 24-hour composites taken daily and held for discard or analysis depending on later assessment of plant performance.

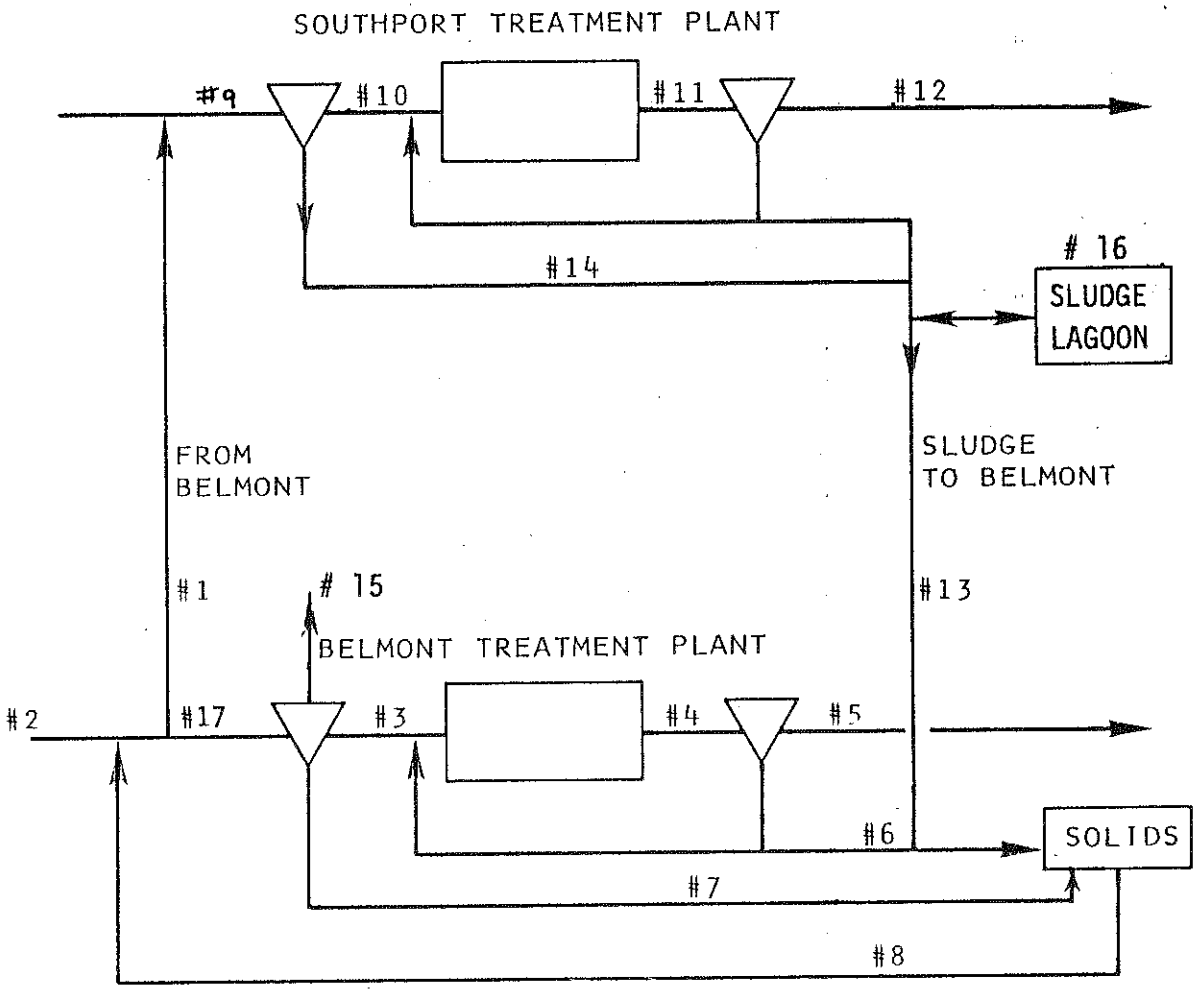
4(A)2.3 OPERATOR REPORTING

Establish operator reporting system. (Integrate with Tasks 8, 9, and 10 monitoring).

4(A)3.1 POTW MASS BALANCE

Collect Priority Pollutant data sufficient to characterize the influent flows to the Southport and Belmont POTW's. Conduct a mass balance around the POTW's to validate the accuracy of the analytical work and to determine the fate of the Priority Pollutants that enter the existing POTW's. Particular attention is to be paid to the performance of the primary treatment system at Belmont, since this system will continue in operation after the startup of the AWT and consequently will not be piloted. Attention is also to be directed toward assessing the importance of the bypass flow of Belmont plant influent to the Southport plant, and of the flow of Southport sludge to the Belmont plant. These flows were not addressed in the previous Burns and Roe characterization and mass balance study (EPA 440/1-79-300). This task requires that Task 4(A)1 be about 50 percent complete, in order that a data base is available. Samples will be analyzed from the Belmont and Southport POTW plant influent, effluent, and recycle streams. The data collected would be utilized to perform a mass balance on the treatment plants and to establish the current loading of conventional and Priority Pollutants on the treatment plant facilities.

The sample locations selected for this task are shown on Figure VI-1. The analytical work in this task consists of Test Battery C and GC/MS analyses for materials that do not biodegrade readily. The normal City POTW monitoring data (BOD, TSS) will be used to establish the balance of conventional pollutants. Table VI-1 presents the schedule of GC/MS and metals sampling and analytical work. Samples will be 24-hour composites prepared by combining grab samples taken manually at three-hour intervals, except where a grab sample is specified in Table VI-1.



TASK 4(A)3
SAMPLING LOCATIONS
AT EXISTING TREATMENT PLANTS
FIGURE VI-1

Sampling Point Descriptions

1 - Wastewater Pumped from Belmont to Southport
2 - Belmont Influent
3 - Belmont Primary Effluent
4 - Belmont Aeration Effluent
5 - Belmont Clarifier Effluent to River
6 - Belmont Sludge - Waste Activated
7 - Belmont Sludge - Primary
8 - Belmont Filtrate and DAF Subnatant Composite
9 - Southport Influent
#10 - Southport Primary Effluent
#11 - Southport Aeration Effluent
#12 - Southport Clarifier Effluent to River
#13 - Southport Sludge to Belmont
#14 - Southport Sludge - Primary
#15 - Belmont Primary Clarifier Scum to Grease Incinerator
#16 - Sludge Lagoon Contents
#17 - Belmont Primary Influent

TABLE VI-1

TASK 4(A)3.1
WASTE CHARACTERIZATION AND MASS BALANCE
SAMPLING AND ANALYTICAL SCHEDULE

Sample Point Number/Name		Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14
2.	Belmont Influent	X,Y		X,Y		X,Y		X,Y		X,Y		X,Y		X,Y	X,Y
9.	Southport Influent	X,Y				X,Y				X,Y		X,Y		X,Y	X,Y
3.	Belmont Prim. Effl.	X,Y		X,Y		X,Y		X,Y		X,Y		X,Y		X,Y	X,Y
5.	Belmont Effl.													X,Y	X,Y
7.	Belmont Prim. Sludge													X,Y	X,Y
6.	Belmont W.A. Sludge													X,Y	X,Y
8.	Belmont Filtrate (S.L.Return) and DAF Subnatant Composite													X,Y	X,Y
12.	Southport Effluent													X,Y	X,Y
13.	Southport Comb. Sludge													X,Y	X,Y
	Belmont Prim. Infl.													X,Y	X,Y
10.	Southport Prim. Effl.													X,Y	X,Y
16.	Lagoon Sludge												X,Y,Z		

X = Test Battery C Y = GC/MS Z = Grab Sample

Based on preliminary results from the sludge study being performed by another contractor, 14 days of sampling appears to have been sufficient to provide for an adequate seven-day solids balance. In keeping with the intention expressed in the plan of study (page IV.22) to maximize the cost effectiveness of both the sludge study and the pretreatment study, the pretreatment project team will attempt to perform a heavy metals balance around the Belmont and Southport plant using the metals data gathered by the sludge study contractor, supplemented by data gathered routinely by the City. This balance will be checked by recalculating it based on metals analyses run on 24-hour composite pretreatment program samples taken over a two-day period as shown in Table VI-1.

Once a calibrated metals balance has been formulated, GC/MS data on priority organics taken simultaneously with the pretreatment program metals samples will be balanced, using the same flows and calibration factors. These GC/MS samples are also shown in Table VI-1.

Prior to the two-day mass balance sampling effort, metals and GC/MS analyses will be run on 24-hour composites from the Belmont and Southport influent flows, and from the Belmont plant primary effluent, as shown in Table VI-1. These samples will be taken every other day over a two-week period, in order to produce an initial characterization of the Indianapolis wastewater. The Belmont primary effluent is to be tested because it will form the influent to the pilot plant. The sampling frequency and period length are selected to spread the budgeted analytical work over as long a period of time as possible while assuring that the samples taken will be representative of the wastewater entering the POTW's. Less frequent sampling allows a better than 50 percent probability

that short (eight-hour) industrial slugs may pass through undetected. Continuous daily sampling increases the probability of detecting an industrial slug less than it increases costs.

Running the influent characterization portion of this task just prior to the two days of mass balance sampling minimizes the chances of having a major industrial slug from a previous week upset the mass balance by causing high retained concentrations in sludge when the influent values are low.

The results of the analytical tests run in Task 4(A)3.1 will be available for evaluation about 20 days after the completion of sampling. At this time, an attempt will be made to formulate an abbreviated GC/ab analytical procedure to track the majority of the compounds of interest through the pilot plant work. As an example, since the Purge and Trap GC/MS test for volatile organics will detect more than half of the compounds detected by Burns and Roe in their 1978 study of the Belmont influent (EPA-440/1-79-300), and since these compounds are the most likely to air strip, the volatiles test might be appropriate for testing oxygen activated sludge influent and effluent. Similarly, the GC/ECD scan run by EMS labs is designed to detect the halogenated compounds (pesticides herbicides) that tend to be among the most toxic of the Priority Pollutants, so this test is appropriate for attempts to quickly identify the compound responsible for bioassay toxicity. However, since the standard JMM GC/MS analytical Priority Pollutant package is already designed to be the minimum testing necessary to identify and quantify all the organic Priority Pollutants, this procedure must be relied upon whenever identification of all Priority Pollutants present is required.

4(A)3.2 POTW BIOASSAYS

Conduct bioassay studies on the influent and effluent from the existing POTW's during the waste characterization period to characterize the general toxicity of the wastewater, to establish the potential impact of industrial waste toxicity on the river, and to document any affects on the POTW's. The testing would be done in connection with the mass balance and upset analysis testing to maximize the use of data collected. The testing is to be done prior to the pilot plant bioassay work to aid in the planning of the latter. The testing will deal primarily with the influent to the POTW rather than the effluent. The tests include 48-hour acute toxicity test using daphnia, 96-hour toxicity tests using fathead minnows, 28-day Daphnia life-cycle tests, and 32-day embryo-larval tests with minnows. If high ammonia acute toxicity precludes effective life cycle test, other tests will be substituted. Attempts will be made to assay samples from which ammonia has been selectively removed, or in which ammonia toxicity is reduced by pH adjustment. See section VII-D for additional discussion of bioassay procedures.

Samples would be collected and analysed to identify the constituents that contribute to the toxicity. Test Battery A would be used in the analyses of these samples, in addition to the daily bioassay monitoring analyses (D.O, temp., pH, Mg, Ca, Hardness, CO_2 ,Alkalintiy, Acidity) that are considered part of running a bioassay test. Also, five of these samples are to be analysed by GC/MS, after having been show to exhibit significant toxicity. This testing program would thus collect data necessary to characterize the wastewater toxicity confirm the results of analyses done for the POTW Mass Balance.

4(A)4.3 PILOT PLANT CONSTRUCTION

Construct the pilot plant. This involves designing the pilot plant, procuring the equipment, erecting the equipment on foundations provided by the City, and starting up the process. (Includes Tasks 4(A)4.1 and 4(A)4.2.) Equipment design is projected to be complete three months after project initiation. Equipment deliveries will begin with the UNOX trailer two and one-half months after project initiation and will be essentially complete by the middle of the fifth month. Process startup will begin with operation of the UNOX plant by the end of the third month and all units will be running under design conditions after five months. (See the attached draft of Chapter V of the Pilot Plant Report, entitled Pilot Plant Design and Construction.)

4(A)4.4.1 TOLERANCE TESTING

Conduct tolerance testing to determine the detrimental effects on the AWT of any Priority Pollutants in the City wastewater. This will be done in two parts. In Part 1, the pilot plant shall be run at design conditions for long enough to collect one month's steady-state data representing normal Priority Pollutant tolerance and removal. Any upsets or unusual conditions will be correlated with analytical results. In Part 2, spikes of selected Priority Pollutants will be introduced into the pilot plant influent, and the effects monitored. Organic removal rate, effluent suspended solids concentrations, nitrification efficiency, and sludge settling characteristics will be monitored. Spike pollutants will be selected based on their presence in the Indianapolis wastewater, and the engineers estimate of their potential for causing treatment problems. If possible, a spike solution will be formulated containing all of the priority organics found in the Belmont influent at one time.

In general, spike pollutants will be introduced at concentrations two to five times those detected in influent samples to magnify any effects and to increase analytical accuracy when measuring removal rate. This task is run simultaneously with Task (A)4.4.2, and will take at least two months.

The operating conditions planned for each of the treatment units during design case run and spike tests are detailed in the schedule on Table V-3.

Analytical work for Tasks 4(A)4.4.1 (and 4(A) 4.4.2, 4(A)4.4.5, and 4(A)4.4.6) includes the following:

- Operational monitoring tests such as SVI, D.O., O_3 dose, pH, and temperature are run daily by the JMM pilot plant operators. See Table V-4.

- Conventional pollutant analyses (Test Battery E) are run on pilot plant influent, effluent, trickling filter effluent, activated sludge effluent, and granular media filter effluent once each day. These are run by the city Belmont laboratory.

- Conventional and Priority Pollutant analyses (Test Batteries B and D) are run on 400 selected samples during the course of the 11-month pilot run. Between two and five samples per day will be run during significant periods of pilot plant operation. A "significant" period is, for example, one during which the pilot plant is fully acclimated to a set of operating parameters, or is subjected to a spike loading of a Priority Pollutant. Periods during which the plant is acclimating to changed operating parameters or recovering from a spike load are not "significant" and are only lightly monitored.

- o GC/MS analyses and GC scans for organic Priority Pollutants will be run on selected samples during significant periods of pilot plant operation. Reliance is placed on complete GC/MS analysis to identify unknown materials in the pilot plant influent and the breakdown products from partially biodegraded spike loading compounds, while simplified GC or GC/MS procedures may be used to monitor previously identified waste constituents or know spike loading compounds.

4(A)4.4.2 REMOVAL TESTING

Conduct removal evaluation testing to determine the capability of the AWT for removing Priority Pollutants. This task will be accomplished at the same time as Task 4(A)4.4.1. Part 1 will be a pilot plant run at design conditions, with Priority Pollutant analyses run at various points in the process.

Part 2 will consist of running pollutant spikes through the pilot plant. Some of the spike pollutants will be those chosen for Task 4(A)4.4.1, while others may be chosen because of the likelihood of high or low removal in the plant. This task is run simultaneously with Task 4(A)4.4.1, and the analytical work is the same as for the latter task.

4(A)4.4.3 OFF-GAS ANALYSIS

The off-gases from the trickling filter and pure oxygen activated sludge plant will be analyzed to determine whether stripping plays a significant role in the removal of volatile Priority Pollutants. Using a personal air monitor, off-gas will be pumped through a Colb Tenax GC trap. The trap will be sealed and sent to the laboratory, where it will be purged and

analyzed on the GC/MS unit, using the same procedures as for volatile organic Priority Pollutant analysis.

4(A)4.4.4 PILOT EFFLUENT BIOASSAYS

Bioassays will be run on the pilot plant effluent, to quantify the removal of toxicity through the AWT. One 32-day embryo-larval test and one 28-day life cycle test will be run while the pilot plant is running under design conditions. Other 48-hour static and 96-hour continuous bioassays will be run at appropriate times, such as when the pilot plant is subjected to a pollutant spike. In addition to the normal bioassay monitoring tests included in the bioassay procedure, selected tests from Test Battery A will be run to check water quality parameters suspected of influencing toxicity. Tissue analyses using Test Battery D and GC/MS will be run on test animals to check for bioaccumulation. By determining the toxicity of the Indianapolis effluents as it will be when the AWT plants start up, these bioassays will provide technical support for increased pretreatment, or indicate that the current levels of industrial waste discharge are acceptable.

4(A)4.4.5 OPERATIONAL ENHANCEMENT

Operational techniques for enhancing removal of Priority Pollutants (or conventional pollutants) will be tested. Part 1 of this task calls for testing of several ozone dosages while monitoring both coliforms and priority organic compounds. This test will be run on half of the treated wastewater flow, while the other half is operated on design dosage and run to the bioassay. Part 2 of this task will investigate the effects of varying pH levels in the activated sludge plant, varying air flows in the trickling filter, and varying solids retention time (SRT) in the Unox plant.

The pH level and filter air flow studies will be run concurrently first, and then the SRT studies will be run. Running the two tests simultaneously will make the most efficient use of running time, and the effects on AWT performance should be separately identifiable since different treatment units are involved.

By conducting these tests in the pilot plant, there will be minimal cost and no risk of full-scale upset compared to testing on a full-scale treatment plant. The results of this testing could lead to improved pollutant removals by the treatment facilities. The analytical work in this task includes Test Battery A and GC/MS scans.

The operating conditions planned for each treatment unit during the operational enhancement experiments are detailed on the schedule in Table V-3.

4(A)4.4.6 DESIGN MODIFICATIONS

Proposed design modifications for enhanced removal of Priority Pollutants will be tested, generally on an off-line or side-stream basis (Part 1). For example, the potential of PAC or primary lime precipitation treatment can be checked with jar tests, while tertiary carbon columns or lime precipitation tests can be run on half of the pilot plant flow. This avoids irreversibly upsetting the pilot UNOX operation and allows maintenance of a flow of treated wate to the bioassays.

Part 2 of this task would consist of a one month trial of an on-line design modification to be determined during the course of the study. Alternatively, Part 2 may be dropped in favor of additional spike tolerance and removal testing.

If evaluation of any design modifications indicate increased pollutant removals, recommendations would be made for further investigations and possible implementation in the treatment system. The incorporation of a process which improves pollutant removals could directly affect the basis of a sewer use ordinance and is therefore an important consideration in the pilot plant program. The results of this task may be used in Task 14 to evaluate joint pretreatment alternatives. The analytical work in this task includes Test Battery A and GC/MS scans.

4(A)4.4.7 PILOT PLANT REPORT

The pilot plant report will be prepared during the last month of operation for submittal to the City soon thereafter. In addition, as each phase of the pilot plant operation is completed, a draft report section will be prepared for inclusion in the monthly report to the City. Table VI-2 shows the preliminary outline for the Pilot Plant Report. (See also the attached draft of Chapter V of the report.)

4(A)5.1 WATER QUALITY DATA REVIEW

Review the available water quality data for the White River. Particular attention shall be paid to previous fish tissue analyses, previous organic and inorganic Priority Pollutant analyses, and other work that will aid in documenting the impact of Priority Pollutants on the river. In the event that the available data is insufficient to support the water quality modeling to be done in Task 15, water samples would be collected from above and below the Belmont and Southport discharges, and analyzed for the full spectrum of Priority Pollutants (Sampling and Analytical work is deferred).

TABLE VI-2

PILOT PLANT REPORT

PRELIMINARY OUTLINE/TABLE OF CONTENTS

I	Introduction
II	Results and Conclusions
III	Wastewater Characterization and Mass Balance
IV	Treatment Plant Upset Analysis
V	Pilot Plant Design and Construction
VI	Tolerance and Removal Evaluation
VII	Operational Removal Enhancement
VIII	Removal Enhancement By Design Modification
IX	Existing River Water Quality Data Review
X	River Sampling and Analytical Results
XI	Influence of Priority Pollutants on River Water Quality
XII	Influence of Priority Pollutants on AWT Performance
XIII	Technical Input to Industrial Waste Ordinance

4(A)5.5 TISSUE ANALYSES

This task involves the analysis of fish tissue samples collected upstream and downstream of the treatment plants. Tissue analyses would include Test Battery D and GC/MS scans. This data would characterize the effects of the treatment plant discharges and the combined sewer overflow on fish in the White River. This task would support the development of a sewer use ordinance designed to protect against bioaccumulation of toxic pollutants in the aquatic life in the White River. (Deferred)

4(A)5.6 PEAK FLOW BIOASSAYS

96-hour flow-through and 24-hour acute bioassay tests will be conducted on samples of peak combined sewer flow. Analysis of the combined sewer flow would be conducted if acute toxicity is displayed. This task would establish sewer use limitations for control of acute toxicity from the combined sewer overflows. Analytical testing includes Test Battery A and GC/MS scans. GC/MS scans would also be conducted on two tissue samples from bioassay organisms. (Deferred)

4(A)5.7 SEDIMENT ANALYSES

Sediment samples from the White River will be analyzed to determine whether Priority Pollutants are accumulating in the benthos. Upstream and downstream samples will be analyzed for inorganic and biological accumulation, using Test Battery A and GC/MS. The results of this testing will support sewer use limitations on Priority Pollutants that accumulate in the sediment.

4(A)5.10 WATER QUALITY REPORT

Prepare a water quality report, summarizing the results of sediment sampling, existing water quality data review and any other analytical work performed in this study. This report will form part of the Pilot Plant Report, as shown in the outline in Table VI-1.

4(A)6 ORDINANCE SUPPORT DOCUMENT

Prepare a sewer use ordinance support document, incorporating the results of both the pilot plant and water quality studies. This ordinance support document will include all of the technical information developed during the course of Task 4(A) as well as other tasks (e.g., Tasks 3, 5, 15) that is useful in the development and justification of the new Industrial Waste Ordinance.

3.8 INDUSTRIAL DISCHARGE SAMPLING

While not part of Task 4, this task includes sampling and analytical work. This task involves the analysis of industrial discharges for Priority Pollutants where data is lacking or is insufficient to characterize the nature of the discharge. The need for such sampling and analysis would be established following a review of the existing data on industrial sewer users. Test Battery A would be utilized in the analysis of these samples. (Deferred)

VII SAMPLING AND ANALYTICAL PROGRAM

A. Summary of Sampling and Analytical Work:

Task	Description	No. Samples	Analysis**	
4(A)2.2	Treatment Plant Upset Sampling and Analysis	25	Test Battery A	EMS
		3	GC/MS	JMM
4(A)3.1.2	Waste Characterization and Mass Balance	40	Test Battery C +CN + Phenols	EMS
		40	GC/MS	JMM
		2	Asbestos	JMM
			Additional Sample as Required	JMM +EMS
4(A)3.2	Treatment Plant Influent and Effluent Bioassay	29 Tests	Static, Acute 48-Hour Tests w/Daphnia	EMS
		5 Tests	Static, Acute 48-Hour Tests w/Daphnia During Upsets	EMS
		2 Tests	Continuous, 96-Hour w/Minnows	EMS
		1 Test	28-Day Daphnia Life Cycle	EMS
		15	Test Battery C or A	EMS
		5	GC/MS	JMM
			Additional Confirmation Samples as Required	EMS
4(A)4.4.1	Pilot Plant Tolerance Testing	400	Test Battery B	JMM
		150	Test Battery C	JMM
		100	GC/ECD	JMM
		20	GC/MS	JMM
		5/Day	Test Battery E	By City for Duration of Task 4(A)4

*Test effort may be shifted to pilot plant effluent if NH_3-N toxicity masks other effects in existing influent.

** See Table VII-I.

Task	Description	No. Samples	Analysis**	
4(A)4.4.3	Off-Gas Analysis	50	GC/MS (Volatiles)	JMM
4(A)4.4.4	Pilot Plant	5 Tests	48-Hour Static	EMS
	Bioassay Tests	5 Tests	96-Hour Continuous w/Minnows	EMS
		2 Duplicate Tests	Continuous Flow 32-Day, w/Minnows	EMS
		2 Tests	Continuous Flow 28-Day Life Cycle with Daphnia	EMS
		10 Tissue	Test Battery D	JMM
		10 Tissue	GC/MS	JMM
		50	Selected Tests from Test Battery A	EMS
4(A)4.4.5	Operational Changes	25	Test Battery A	EMS
		10	GC/MS	JMM
4(A)4.4.6	Design Modifications	10	Test Battery A	EMS
		5	GC/MS	JMM
4(A)5.1*	Upstream and Downstream River Water Quality	50 15	Test Battery A GC/MS	JMM JMM
4(A)5.5*	Fish Tissue	20	Test Battery D GC/MS	JMM JMM
4(A)5.6*	CSO Storm Water	30 Tests	48-Hour Static	EMS
	Bioassay	10 Tests 20 Tests 5 2 Tissue	96-Hour Continuous Test Battery A GC/MS GC/MS	EMS EMS JMM JMM
4(A)5.7	River Sediment	25	Test Battery A	JMM
	Samples	10	GC/MS	JMM
3.8*	In-Sewer Sampling	To Be Determined	Test Battery A	

*Deferred tasks.
**See Table VII-I.

TABLE VII-1

TEST BATTERY A

Metals

Cadmium	Total Kjeldahl Nitrogen
Nickel	Fats, Oil, and Grease
Copper	Cyanide
Chromium	Phenols
Zinc	pH
Lead	Total Suspended Solids
Mercury	BOD
Ammonia Nitrogen	COD
NO_3 -N	GC/ECD Scan

TEST BATTERY B

TKN	Cyanide
Fats, Oils, and Grease	Phenols
COD (total)	
TOC (soluble)	

TEST BATTERY C

Cadmium	Zinc
Nickel	Lead
Copper	Mercury
Chromium	Berillium
Arsenic	Selenium
Antimony	Thallium
Silver	

TEST BATTERY D

Cadmium	Zinc
Nickel	Lead
Copper	Mercury
Chromium	

TEST BATTERY E

BOD (total + soluble, inhibited)	NH_3-N
COD (total)	NO_3-N
Total Suspended Solids	No_2 -N

B. List of Analytical Methods: JMM and EMS

LIST OF JMM ANALYTICAL METHODS

PRETREATMENT PROGRAM

INDIANAPOLIS

Table 2 below summarizes the parameters, methods, and references to be used by the JMM-ERL during the Indianapolis pretreatment study:

TABLE 2

JMM-ERL ANALYTICAL METHODS

PARAMETER		METHOD	REFERENCE
Color	a)	Platinum-cobalt	EPA[1] 110.2
pH	a)	Glass electrode	EPA 150.1
Residue, filterable	a)	Gravimetric	EPA 160.1
Aluminum	a)	ICP[4]	Fed. Reg. Vol. 44(233) = 69559
Antimony	a)	Graphite furnace	EPA 204.2
Arsenic	b)	Graphite furnace	EPA 206.2
Barium	a)	ICP	
Beryllium	a)	ICP	Fed. Reg. Vol. 44(233) = 69559
Boron	a)	ICP	Fed. Reg. Vol. 44(233) = 69559
Cadmium	a)	Atomic absorption	EPA 213.1

TABLE 2 (continued)

PARAMETER		METHOD	REFERENCE
Chromium	a)	ICP	Fed. Reg. Vol. 44(233) = 69559
Cobalt	a)	Graphite furnace	EPA 219.2
Copper	a)	ICP	Fed. Reg. Vol. 44(233) = 69559
Iron	a)	Atomic absorption	EPA 236.1
Lead	a)	Graphite furnace	EPA 239.2
Magnesium	a)	ICP	Fed. Reg. Vol. 44(233) = 69559
Manganese	a)	ICP	Fed. Reg. Vol. 44(233) = 69559
Mercury	a)	Cold vapor, manual	EPA 245.1
Molybdenum	a)	ICP	Fed. Reg. Vol. 44(233) = 69599
Nickel	a)	ICP	Fed. Reg. Vol. 44(233) = 69599
Selenium	a)	Grapite furnace	EPA 270.2
Silver	a)	Atomic absorption	EPA 272.1
Thallium	a)	Graphite furnace	EPA 279.2
Tin	a)	ICP	Fed. Reg. Vol. 44(233) = 69599
Titanium	a)	ICP	Fed. Reg. Vol. 44(233) = 69599

TABLE 2 (continued)

PARAMETER		METHOD	REFERENCE
Zinc	a)	ICP	Fed. Reg. Vol. 44(233) = 69599
Bromide	a)	Ion chromatography	USGS[3]
Chlorine Residual (on site)	a)	DPD method, Colorimetric	Std Mthd 409F
Cyanide	a)	Colorimetric, pyridine-pyrozolone	EPA 335.2
Fluoride	a)	Alizarin fluoride blue, automated	EPA 340.3
Nitrogen, Ammonia	a)	Colorimetric, automated phenate	EPA 350.1
Nitrogen, Total Kjeldahl	a)	Colorimetric, semi-automated block digester AAII	EPA 351.2
Nitrogen, Nitrate	a)	Cadmium reduction, automated	EPA 353.2
Nitrogen, Nitrite	a)	Cadmium reduction, automated	EPA 353.2
Oil and Grease	a)	Gravimetric	EPA 413.1
Oxygen, BOD	a)	5 day, 20 C	EPA 405.1
Oxygen, COD	a)	Titrimetric	EPA 410.1
Phenol	a)	Spectrophotometric, manual	EPA 420.1
Phosphorus-total	a)	Colorimetric, automated, block digestor, AAII	EPA 365.4
Sulfate	a)	Methyl thymol blue, automated	EPA 375.2
Sulfide	a)	Titrimetric, iodine	EPA 376.1

TABLE 2 (continued)

PARAMETER		METHOD	REFERENCE
Sulfite	a)	Titrimetric, potassium iodide-iodate	EPA 377.1
Surfactants	a)	Methylene blue	EPA 425.1
Base/Neutral and Acid Extractibles	a)	GC/MS	EPA 625
Total Organic Carbon	a)	Combustion or oxidation	EPA 415.1
Volatile Organics	a)	Purge and trap, GC/MS	EPA 624
Gross Alpha and Beta Radiation	a)	Proportional Counter	Std. Mthd. 703
Fecal Coliforms	a)	Most Probable Number	Std. Mthd. 908

1) EPA, Methods for Chemical Analysis of Water and Wastes, 1979.

2) APHA, Standard Methods, 14th Edition, 1975.

3) USGS, WRI-79-101.

4) Inductively Coupled Radiofrequency Plasma Source (ICP) for emission spectroscopy via the Perkin-Elmer ICP/5000 system.

5) Atomic Absorption Spectrometry (AAS) via a Perkin-Elmer 305B, which consists of an automated HGA 2200 graphite furnace and a cold vapor mercury attachment and via a PE5000 with an HGA furnace, also automated.

Chemical Analysis - Protocols : EMS

The methods of analysis used by EMS Laboratories, Inc. conform to those methods current approved by the USEPA. In addition, the company offers other types of tests and testin capabilities. Table 1 lists the methods of analysis to be utilized during the pretreat ment program as well as the corresponding EPA method code.

Methods for sampling, sample preservation and sample storage and holding times will con form to those protocols listed in the Federal Register, Vol 44, No 233; Monday, Decembe 3, 1979 pages 69464 - 69575

Table 3

EMS ANALYTICAL METHODS

Parameter	Method
Cadmium	Flame Atomic Absorption (EPA 213.1)
Nickel	Flame Atomic Absorption (EPA 249.1)
Copper	Flame Atomic Absorption (EPA 220.1)
Chromium	Flame Atomic Absorption (EPA218.1)
Zinc	Flame Atomic Absorption (EPA 289.1)
Lead	Flame Atomic Absorption (EPA 239.1)
Mercury	Flameless Atomic Absorption (EPA 245.1)
Ammonia Nitrogen	Ion Selective Electrode (EPA 350.3)
Kjeldahl Nitrogen	Bloch Digester/Electrode (EPA 351.4)
Oil & Grease	Liquid - Liquid Extraction (EPA 413.1)
Cyanide	Automated Barbitaric Acid (EPA 335.3)
Phenols	Automated 4 AAP (EPA 420.2)
pH	Electrode (EPA 150.1)
Suspended Solids	Glass fiber filtration (EPA 160.2)
BOD	Probe (EPA 405.1)
COD	Dichromate reflux (EPA 410.1)
GC/ECD Scan	SE-54 Capillary Column, 25 M, (EPA 608)
Total Solids	Gravimetric (EPA 160.3)
Total Volatile Solids	Gravimetric (EPA 160.4)
Hardness	Titrimetric (EPA 130.2)
Alkalinity	Titrimetric to pH 4.5 EPA
Acidity	Titrimetric to pH 8.2 (EPA 305.1)
Calcium	Flame Atomic Absorption (EPA 215.1)
Magnesium	Flame Atomic Absorption (EPA 242.1)
Sample Presp for AA	"EPA Metals" pp Metal 1 - Metals 19

*GC/ECD Conditions can be adjusted based upon compound(s) to be targeted. However, standard GC/ECD scan will report the following compounds:

Aroclor 1242	Endrin	Aldrin
Aroclor 1254	Lindane	Dieldrin
Aroclor 1248	Methoxychlor	4,4 DDT
Aroclor 1260	Toxaphene	
Aroclor 1016	Heptachlor	

Other chromatographic peaks will be reported and chromatographic conditions will be included in the report.

C. COLLECTION, PRESERVATION AND STORAGE OF WATER SAMPLES

A gallon sample should suffice for most physical and chemical analyses. No attempt should be made to use the same sample for chemical, bacteriological and microscopic examination because the methods of collection and handling are quite different.

DETERMINATION	CONTAINER	MINIMUM SAMPLE SIZE	PRESERVATION	MAXIMUM HOLDING PERIOD
Acidity	*Plastic or pyrex bottle Tightly sealed, no bubbles	100 ml	Refrigeration at 4°C	14 days
Alkalinity	Plastic or pyrex bottle Tightly sealed, no bubbles	100 ml	Refrigeration at 4°C	14 days
Aluminum	Plastic or glass HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Arsenic	Plastic or glass HNO_3 rinse	100-200 ml	HNO_3 to pH 2	6 months
Bacteria, Coliform	Steriled plastic or glass	100-1000 ml	Cool, 4°C, $Na_2S_2O_3$	6 hours
Barium	Plastic or glass HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Beryllium	Plastic or glass HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Boron	Plastic HNO_3 rinse	50-10 ml	None required	6 months
Bromide	Plastic or glass HNO_3 rinse	250-500 ml	None required	28 days
Cadmium	Plastic or glass HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Calcium	Plastic or glass HNO_3 rinse	100-250 ml	HNO_3 to pH 2	6 months
Carbon Dioxide (Free)	Plastic tightly sealed, completely filled	500 ml and overflow the bottle	Preserve at lower temperature	Titrate at site or measure pH at site and do alkalinity at lab.
Chloride	Plastic or glass	50-20 ml	None required	28 days
Chlorine (Residual)	Glass	200-500 ml	Avoid sunlight or other strong light; refrigerate at 4°C	2 hrs.
Chlorine (Demand)	Plastic or glass	4000 ml	Freeze	3 days
Chromium (Hexavalent)	Unscratched glass or plastic bottle HNO_3 rinse	100-200 ml	Refrigeration at 4°C	2 days
Chromium (Total)	Glass or plastic bottle HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Cobalt	Plastic or glass HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Color	Clean glass, plastic	100 ml	Refrigeration at 4°C	2 days
Copper	Plastic or glass HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Cyanide	Plastic or glass	500-1000 ml	NaOH to pH 12 and refrigerate at 4°C 0.008% $Na_2S_2O_3$ (if chlorinated)	14 days

TABLE 4

COLLECTION, PRESERVATION AND STORAGE OF WATER SAMPLES
(CONTINUED)

DETERMINATION	CONTAINER	MINIMUM SAMPLE SIZE	PRESERVATION	MAXIMUM HOLDING PERIOD
Detergents		See surfactants		
Fluoride	Plastic	300-500 ml	None required	28 days
General Mineral (Survey) Analysis	Plastic or glass	4000 ml	Keep at 15°C	3 days
Grease		See oil and grease		
Hardness	Plastic or glass	50-100 ml	HNO_3 to pH 2	6 months
Iodide	Plastic or glass	100 ml	None	28 days
Iron Total	Glass or plastic HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Lead	Plastic or glass HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Lithium	Pyrex or glass bottle	100 ml	None required	6 months
Magnesium	Plastic or glass HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Manganese	Glass or plastic HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Metals Total (in general)	Glass or plastic HNO_3 rinse	1000 ml	HNO_3 to pH 2	6 months
Metals Dissolved (in general)	Glass or plastic HNO_3 rinse	500 ml	Filter through 0.45 membrane filter and HNO_3 to pH 2	6 months
MBAS (Methylene Blue Active Substances)		See Surfactants		
Mercury	Glass or plastic HNO_3 rinse	500 ml	HNO_3 to pH 2 0.05% $K_2Cr_2O_7$	28 days
Nickel	Plastic or glass HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Nitrogen-Nitrite/ Nitrate	Glass or plastic	100-250 ml	H_2SO_4 to pH 2 Cool, 4°C	28 days
Nitrogen (Ammonia)	Glass or plastic	1000 ml	H_2SO_4 to pH 2 Cool, 4°C	28 days
Nitrogen (Nitrate)	Glass or plastic	100-250 ml	Cool, 4°C	2 days
Nitrogen (Nitrite)	Glass or plastic	100-250 ml	Cool, 4°C	2 days
Nitrogen (Organic)	Glass or plastic	1000 ml	Refrigeration at 4°C	28 days
Nitrogen, Kjeldahl (Total)	Glass or plastic	500-800 ml	H_2SO_4 to pH 2	28 days
Organic Carbon	Glass, teflon liner	50 ml	Cool, 4°C	28 days
Odor	Odor-free glass, no air	500 ml (completely filled)	Refrigeration at 4°C	24 hours

TABLE 4

COLLECTION, PRESERVATION AND STORAGE OF WATER SAMPLES
(CONTINUED)

DETERMINATION	CONTAINER	MINIMUM SAMPLE SIZE	PRESERVATION	MAXIMUM HOLDING PERIOD
Oil and Grease	Widemouth glass Solvent rinse Teflon liner	800 ml	2 ml H_2SO_4/ liter at 4°C pH 2 Sludge sample; 1 ml Conc H_2SO_4/80 gm of sludge	28 days
Oxygen, Dissolved (DO)	Glass or plastic	300 ml Fill to neck	Determine on site	1 hour
Oxygen Demand, Biochemical (BOD)	Glass or plastic	1000-2000 ml	Cool, 4°C	2 days
Oxygen Demand, Chemical (COD)	Glass or plastic	100-500 ml	H_2SO_4 to pH 2 Refrigerate at 4°C	28 days
pH	Glass or plastic	100 ml	None	2 hours
Phenolics	Glass	500-1000 ml	H_2SO_4 to pH 2 Cool, 4°C	28 days
Phosphorus (all forms)	Glass	500 ml	Freezing or 40 mg $HgCl_2$/1 and refrigerate at 4°C	7 days
Phosphate (Total)	Glass or plastic HCL rinse	50-250 ml	H_2SO_4 to pH 1.5 Refrigerate at 4°C	28 days
Potassium	Plastic or pyrex bottles	100-250 ml	HNO_3 to pH 2	6 months
Residue		See solids		
Selenium	Glass or plastic HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Silica	Plastic	100 ml	Refrigerate at 4°C	28 days
Silver	Plastic HNO_3 rinse	200-300 ml	HNO_3 to pH 2	30 days
Sodium	Plastic or glass HNO_3 rinse	100-250 ml	HNO_3 to pH 2	6 months
Solids	Plastic or resistant glass bottle	500-1000 ml	Refrigerate at 4°C	7 days
Strontium	Plastic or pyrex bottle	100 ml	HNO_3 to pH 2	6 months
Sulfate	Glass or plastic	100-500 ml	Refrigeration at 4°C	28 days
Sulfite	Glass or plastic Tightly sealed, no bubbles	200-500 ml Fill to neck	Refrigerate at 4°C	48 hours
Sulfide Total	Glass or plastic	500 ml (no aeration) Fill to neck	Field analyses or 2 ml Zn acetate/liter and refrigerate at 4°C	28 days
Surfactants	Glass or plastic HNO_3 rinse	500-1000 ml	Cool, 4°C	48 hours
Taste	Glass	500 ml	Refrigeration at 4°C	48 hours
Turbidity	Glass or plastic	100 ml	Refrigeration at 4°C; store in dark	4 hours

TABLE 4

COLLECTION, PRESERVATION AND STORAGE OF WATER SAMPLES
(CONTINUED)

DETERMINATION	CONTAINER	MINIMUM SAMPLE SIZE	PRESERVATION	MAXIMUM HOLDING PERIOD
Specific Conductance	Glass or plastic	100 ml	Cool, 4°C	28 days
Vanadium	Glass or plastic HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Zinc	Glass or plastic HNO_3 rinse	200-500 ml	HNO_3 to pH 2	6 months
Sludge and Bottom Sediment	Plastic bottles of 120 cc size	120 cc	5 gm Sodium benzoate or 1 ml conc H_2SO_4/80 gm of sample if no interference with analyses	—
Chlorinated Hydrocarbon Pesticides and PCB's	Glass, teflon liner and solvent rinse or muffled	1000-4000 ml	Cool, 4°C Sediment, sludge sample: Cool 4°C	7 days (until Extraction) 30 days (after extraction)
THM	Glass, teflon-lined cap	60 ml	Cool, 4°C 0.008% $Na_2S_2O_3$	7 days (until Extraction) 30 days (after extraction)
Radiological	Plastic or glass	1000 ml	HNO_3 to pH 2	6 months
ORGANIC COMPOUNDS - GC or GC/MS				
Extractables including phthalates, nitrosamines, organochlorine pesticides, PCB's, PCB's, nitroaromatics, isophorone, polynuclear aromatic hydrocarbons, halo ethers, chlorinated hydrocarbons and TCDD)	G, teflon-lined, cap, muffled	4000 ml	Cool, 4°C $Na_2S_2O_3$	7 days (until extraction) 30 days (after extraction)
Extractables (phenols)	G, teflon-lined cap, muffled	4000 ml	Cool, 4°C $Na_2S_2O_3$ H_2SO_4 to pH 2	7 days (until extraction) 30 days (after extraction)
Purgeables (Halocarbons and aromatics)	G, teflon-lined cap, muffled	60 ml	Cool, 4°C $Na_2S_2O_3$	14 days
Purgeables (Acrolein and Acrylonitrite)	G, teflon-lined cap, muffled	60 ml	Cool, 4°C $Na_2S_2O_3$	3 days

* Plastic bottles are usually of polyethylene; glass bottles are usually of borosilicate.
** No established standards.

VII-D

SUMMARY OF BIOASSAY PROCEDURES INDIANAPOLIS PRETREATMENT PROJECT

The bioassays to be used during the course of the Indianapolis Pretreatment project consist of (1) 48 hour static acute bioassays using daphnia; (2) 96 hour flow through bioassays using fathead minnows and (3) 32 day embro-larval bioassays using fathead minnow embryos and (4) daphnia life cycle tests 20-28 days in duration.

While the original plan of study indicated that approximatley equal emphasis would be placed on pilot plant bioassays and existing POTW bioassays, current plans call for more emphasis to be placed on pilot plant work and a corresponding amount of effort to be removed from existing POTW work. The following discussion sections and TAble D-1 indicate the amount of emphasis to be placed on each area.

Prior to expanding upon the nature of the work to be performed, it is appropriate to first describe the equipment to be used to perform the actual bioassays.

The diluter system to be used is installed in a twenty-foot climate-controlled mobile laboratory. The diluter itself will make seven different dilutions of test water with dilution water. Each of these seven solutions is split into two aquariums for a total of fourteen aquariums. Each aquarium is equipped with two moving egg cups (28 total egg cups) into which smaller test organisms can be placed (eg. daphnia or fish embryos). Water is then supplied to each aquarium on a flow-through basis. See Figure D-1 for a diagram of the diluter system.

EXISTING POTW (4 (A) 3.2)

As currently planned, the waste characterization will consist of 14 days of sampling and analyiss. Concurrent with this phase during the third and fourth

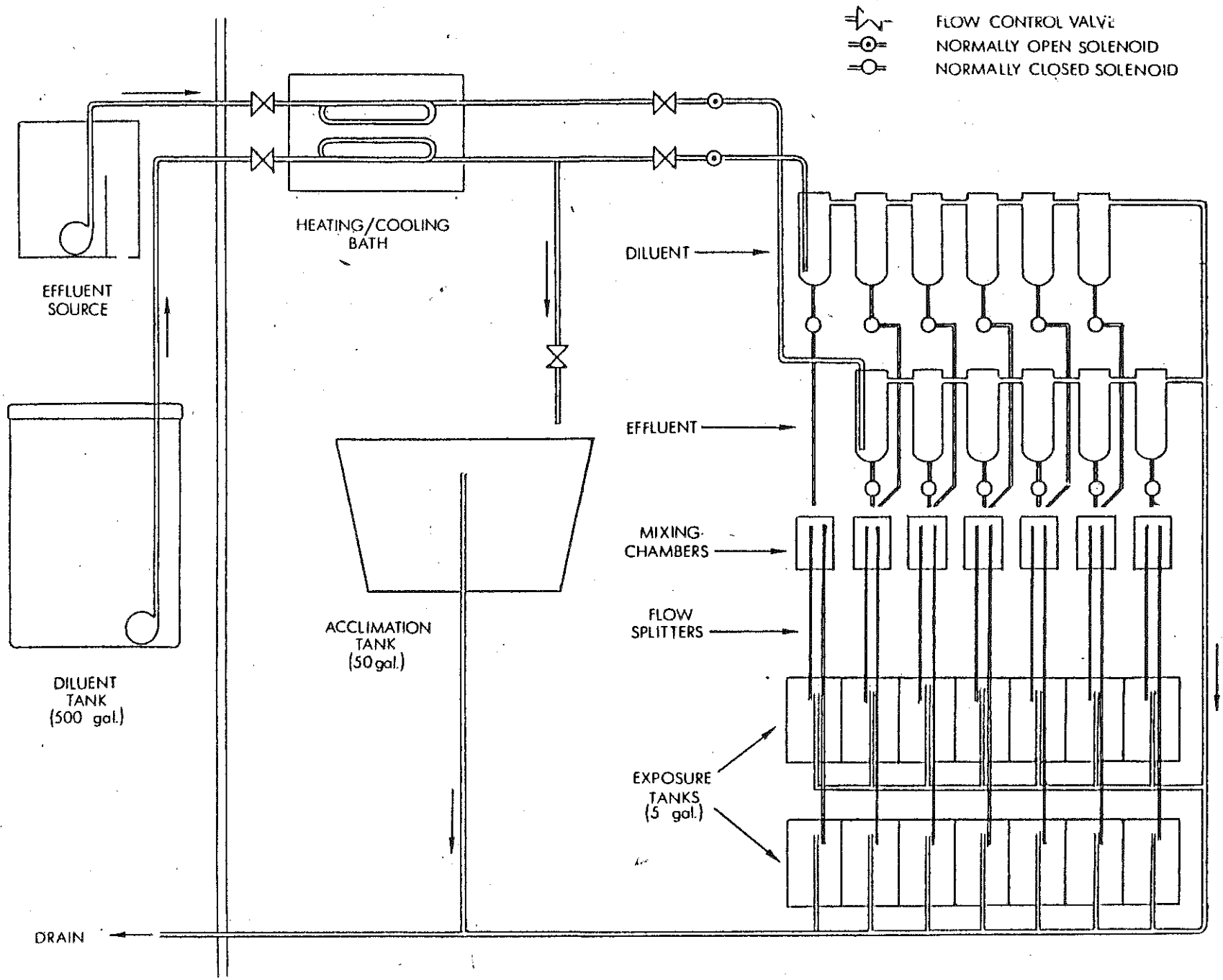


FIGURE D-1

EMS MOBILE BIOASSAY LABORATORY
DILUTER SYSTEM

months of the project, the bioassay diluter system will be run continuously and toxicity to fish and daphnia of the Belmont primary effluent will be monitored on a flow-through basis. Static 48 hour bioassays with daphnia will also be run on the POTW final effluents. The diluter system will continue to be run after the sampling for the waste characterization phase is finished in order to complete a daphnia life-cycle test which is expected to take a total of 20-28 days.

These toxicity tests will be run in conjunction with the chemical analyses in test battery C as well as GC and GC/MS analyses to determine if there is a correlation between the concentrations of any chemical compounds or elements and the toxicity of the POTW primary and final effluents (The primary effluent is stressed because it will be the influent to the new AWT facilities scheduled to go on line in the near future.)

Static bioassays of the influent and final effluent will be conducted on-site as well as off-site. FLow-through tests on the primary effluent will be conducted on-site using the previously described diluter system. Minnows will be placed in aquariums and daphnia will be placed in the associated egg cups in each aquarium. The 48 hour static tests on the final effluents will also take place on-site at Belmont as well as off-site. 48 hour static tests with daphnia will also be performed on alternate days on the final effluents of the Belmont and South port facilities to determine if a correlation exists between the toxicity of the effluent and concentration(s) of any chemical species. Toxicity contributed by plant recycle streams can also be assessed using the data described above.

The information developed will be utilized in several ways. The first way is to determine if there are correlations between toxicity and concentration(s) of chemical species in all test waters. Secondly, a screening procedure will have been developed which the City can ultimately use in determining the effects of a particular industrial effluent on the treatment process. In the absence of extensive chemical analytical data, or in the event of an emergency such as a spill, the City may choose to define acceptability as a graction of the 30 minute

EC50 of a particular waste to a test organism such as daphnia or hathead minnows. In this way, routine and repetitive industrial monitoring for toxics may be simplified and costs greatly reduced. Additionally, the City will be able to respond to emergencies such as spills or other slug loadings in a more confident manner. Thirdly, since many stream standards for specific compounds and elements are defined in terms of the 96 hour LC50, checks to insure complince with stream standrds can be made much more readily and results would be available immediately. Finally, the data generated on the existing POTW is important for the operation of the pretreatment program until the AWT facilities come on line and will assist in the evaluation of the effects of AWT before these facilities become fully operational.

PILOT PLANT BIOASSAYS 4(A)4.4.4

During the operation of the pilot plant, the emphasis will shift from 48 and 96 hours bioassays to 32 day bioassays to assess the sub-acute effect of the pilot plant effluent.

One duplicate 32 day assay will be run while the pilot plant is initially running under design conditions to measure the toxicity of pilot plant effluent with respect to the upstream receiving water. This assay is to be run during the initial phase of pilot plant operations and in conjunction with part 1 of tasks 4(A)4.4.1 and 4(A)4.4.2 to maximize the use of the data collected.

It is now anticipated that an additional duplicate 32 day test will be performed near the end of pilot plant operations to measure the impact of operational enhancements from task 4(A)4.4.5 on the sub-acute toxicity of the pilot plant effluent. Selected tests from test battery A would be performed in this part of the project only as needed.

During both 32 days tests, if possible, life cycle daphnia tests will be run concurently. This will be accomplished by placing the daphnia in the alternate

set of egg cups in each aquarium. 48 hour static tests will also be run. This approach will maximize the data generated while increasing the manpower demand only slightly.

Other 48 hour static and 96 hour flow=through tests would be performed at such times as during and immediately after the introduction of a pollutant spike into the pilot plant. These tests would also be performed on the primary effluent during these periods as required. 96 hour flow-hrough and 48 hour static bioassays will also be performed to assess the effects of operational changes in enhancing the removal of toxics.

All test fish from pilot plant operational bioassays would be saved for tissue analysis using GC/MS and test battery D to test for bioaccumulation in task 4(A)5.5.

The data collected during the pilot plant operations would be used in several ways. First, the toxicity of the AWT effluent would be predicted based upon the results of the two duplicate 32 day embryo-larval tests.

Secondly, the data would be used to check the value of operational changes to enhance the removal of toxics. Thirdly, the impact of pollutant spikes on the toxicity of the AWT effluent could be predicted using 96 hour flow-through tests as well as 48 hour static tests. The acute toxicity of influent and AWT effluent due to spike loadings of pollutants is of concern because of the limited time frame involved in spike or slug loadings. Finally as mentioned earlier, the City would have a tool by which to predict the impact of slug loadings or spills on the operations of AWT.

The following portions of Section VII-D present details of the biossay procedures for the 48-hour static acute daphnia test, the 28-day daphnia life cycle, the 965-hour flow through acute minnow test, and the 32-day embryo-larval minnow test.

TABLE D-1

SUMMARY OF PLANNED BIOASSAYS

Study Phase	Sample Point	of Test per Sample			
		48-Hour Daphnia Acute	28-Day Daphnia Life Cycle	96-Hour Fish, Acute	32-Day Fish Embryo-larval
POTW Waste Characterization	Belmont Effl.	4			
POTW Waste Characterization	Southport Effl.	4			
POTW Waste Characterization	Belmont Infl.	2			
POTW Waste Characterization	Southport Infl.	2			
POTW Waste Characterization	Belmont Prim. Effl.	10	1	2	
POTW Waste Characterization	Southport Prim. Effl.	7			
Pilot Plant Design Case	Pilot Effl.	8	1	2	1 duplicate
Pilot Plant Removal Enhancement or Design Mod.	Pilot Effl.	4	1	3	1 duplicate
Pilot Plant Spikes	Pilot Effl.	4		5	
Upset Analysis	1 Gal Upset Sample, Prim. Effl.	4			

D. BIOASSAY PROCEDURES

PROCEDURE FOR USING DAPHNIA MAGNA IN A 48 HOUR STATIC BIOASSAY

GENERAL

Daphnia magna has been chosen as the invertebrate species to be used in the effluent static bioassay. It is easily cultured and handled in the laboratory and has been used extensively in toxicological studies of both complex effluents and individual substances.

A Parthenogenically reproducing laboratory culture will serve as the supply of test organisms. D. magna have been reared in two 130 liter aquariums using reconstituted water. The animals have been maintained in a constant temperature room at 20 $\pm$ 2 ^{o}C on a diet of a trout chow/yeast mixture recommended in the ASTM "Proposed Standard Practice for Conducting Daphnia magna Chronic Toxicity Tests in a Flow-Through System". A 16 hour photoperiod has been used to stimulate asexual reproduction.

Daphnia are fed a suspension of the food mixture each day, tanks are cleaned once a week and 50 percent of the water is replaced every 2 weeks. Predation is simulated by removing approximately 10% of the population every three days. This helps to avoid overcrowding and ephippia production. Organisms are handled using fire-polished glass tubing and suction bulb.

TEST PROCEDURES

In general, test procedures will follow:

Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms EPA-600/4-78-012

Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians EPA-660/3-75-009

Standard Methods for the Examination of Water and Wastewater, 15th Edition, APHA-AWWA-WPCF

Quality Assurance Guidelines for Biological Testing EPA-600/4-78-043

Daphnia Toxicity Tests
A.L. Buikema, Jr., J.L. Geiger, and D.R. Lee, "Daphnia Toxicity Tests", Aquatic Invertebrate Bioassays, ASTM STP 715, 1980, 48-69.

Acute static tests are conducted using D. magna obtained from the laboratory culture. The Daphnia used in tests less than 48 hours in duration will be first instar neonates. Longer tests may require adults to avoid starvation.

Tests are run in triplicate with controls. Exposure chambers consist of either 250 ml Erlenmeyer flasks or 20 cm Petri dishes, depending upon the effluent concentrations. The proper proportions of effluent and dilution water are determined with a 24 hour range finding test. Concentrations are based on a geometrically spaced series on a log scale.

Daphnia are acclimated to the dilution water one week before the beginning of the test and to test temperature at least 48 hours before the start of the test. Only after the population appears healthy and adapted to the dilution water will they be used in the test.

Neonates less than 24 hours old will be collected by either individually isolating adults with eggs and removing young or by grading the general population through a Nitex screen that has a 0.8 mm mesh size. Neonates will be collected from several separate populations to avoid synchronous molting during the test.

Tests will be conducted at 17 $\pm$ 2 ^{o}C and will be performed in a constant temperature room. A photoperiod of 16 hours light, 8 hours dark will be maintained throughout the test. Observations will be made at 0, 1.5, 3, 6, 12, 24, and 48 hours The number of motile animals will be recorded and an effective concentration or EC_{50} will be reported. At the beginning and end of the test the D.O., pH, alkalinity, hardness, and specific conductance will be recorded. Temperature will be recorded hourly and water samples taken at the beginning and end of the test. At the end of the test, the Daphnia will be transferred to 100% dilution water and observed for 48 hours.

TEST PROTOCOL

10 DAYS PRETEST

Make sure population is in good condition

Check for signs of ephippia (black egg) development. If present check:

1. Overcrowding
2. Adequate feeding (see animal log)
3. Tank cleanliness
4. Water Parameters

    Temperature (20 $\pm$ 2 ^{o}C)
    pH (7.6-8.4)
    Alkalinity (110-245 mg/l as $CaCO_3$)
    Hardness (160-320 mg/l as $CaCO_3$)
    Dissolved Oxygen (> 40% sat.)

5. Photoperiod

7 DAYS PRETEST

Gradually change from 100% holding water to 100% dilution water over a 24 hour period. Make sure D. magna respond well to change and check again for ephippia development. Gradually change holding temperature to test temperature of 17 ^{o}C with no more than 2^{o}C change within 24 hours.

48 HOURS PRETEST

Daphnia must be at 17 $\pm$ 2 ^{o}C and in 100% dilution water with no signs of ephippia. Conduct 24 hour range finding test to determine concentrations necessary for definitive test.

24 HOURS PRETEST

Isolate 70-80 adults with obvious egg development. Transfer from three separate holding tanks using a fire-polished smooth glàss tube and suction bulb. Place in Petri dishes or other suitable clean glass container in groups of 10 per container.

8 HOURS PRETEST

Dilution water and effluent samples must be obtained. Collect effluent and dilution water in a suitable glass container that has been detergent washed, acid rinsed and filtered through a sieve with 2 mm holes. The effluent must not be aerated, agitated or altered in any other way. Both effluent and diluent should be brought to the test emperature of 17 $\pm$ 2 ^{o}C with the aid of a water bath. Dilution water may be aerated to achieve at least 50% saturation of dissolved oxygen.

8 Hours Pretest - Continued

Dilution water should be obtained as near the point discharge as possible, but not in the zone of influence of the waste discharge. Effluent samples can be composite or grab depending on the short and long-term operation of the plant and the variability of the waste.

Measure the hardness, alkalinity, pH and specific conductance of the dilution water.

Temperature must be recorded hourly throughout the test.

4 HOURS PRETEST

Prepare dilutions of effluent and diluent based on the range finding test data. Set up 3 sets of 7 exposure tanks that have been detergent washed, acid rinsed and rinsed with distilled water. Stir the effluent and dilution water samples gently to assure complete mixing and even distribution to the exposure tanks. Combine effluent and diluent in the proper proportions for the test concentrations and final volume. Final volume should be 200 ml per test container.

1 HOUR PRETEST

Remove adults from the nursery containers and combine the young by gently pouring them into one large container making sure not to damage them in transfer. Pour dilutions of effluent into exposure tanks. Select and randomly distribute neonates using a smooth glass tube and suction bulb. Place 10 neonates per test tank but not more than 2 in any one tank at a time. This procedure should be accomplished within 30 minutes. Start temperature recorder or take a reading.

START OF TEST

Start timing test after the last Daphnia is distributed. Observe all exposure tanks and record the number of motile animals in each. Notice immediate vigor and general activity of all Daphnids.

DURING TEST

Record the number of motile organisms at 0, 1.5, 3.0, 6.0, 12, 24, and at 48 hours. Measure the D.O. in any tank where all the Daphnia die. Record any unusual events or characteristics of the Daphnia.

END OF TEST

Record final number of motile animals at 24 (or if possible 48) hours. Remove Daphnids from the exposure tanks. Measure D.O., pH, alkalinity hardness and specific conductance in a control, high, medium and low concentrations. Place test organisms in similar exposure tanks containing 100% dilution water - observe them for 48 hours. Record the number of organisms that show signs of regaining motility and those that do not for each dilution.

"Daphnia sp., 28-day Reproduction Test (including an Acute Immobilisation Test)"

202

In general it is useful to conduct a range-finding toxicity (or acute immobilisation) test (24h EC 50) in preparation for a reproduction test.

The following Test Guideline therefore includes two parts:

Phase I - the 24h EC 50 (acute immobilisation) test

Phase II - the 28-day reproduction test.

PHASE I - RANGE-FINDING 24h EC 50 TEST (Acute Immobilisation)

1. INTRODUCTORY INFORMATION

° Prerequisites

- Water solubility
- Vapour pressure

° Guidance information

- Structural formula
- Purity of the substance
- Methods of analysis for the quantification of the substance in water
- Chemical stability in water and light
- n-Octanol/water partition coefficient
- Results of a test on ready biodegradability (see Test Guidelines 301 A-E)

° Qualifying statement

For chemicals with low solubility under test conditions, it may not be possible to quantitatively determine the EC 50 (see Definitions and units, below).

Users of this Test Guideline should consult the Preface, in particular paragraphs 3, 4, 7 and 8.

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

° Definitions and units

24h EC 50 is the concentration estimated to immobilise 50 per cent of the Daphnia by 24 hours exposure. (If another definition is used, this must be reported, together with its reference.)

Immobilisation: those animals not able to swim another 15 seconds after gentle agitation of the test container are considered to be immobile. (If another definition is used, this must be reported, together with its reference.)

° Reference substances

In the course of the acute immobilisation phase a reference substance may occasionally be tested for EC 50 with the test compound as a means of assuring that the test conditions are reliable. An example of such a useful reference substance is $K_2Cr_2O_7$.

° Principle of the test method

In the acute immobilisation phase of the test varying concentrations of the substance investigated exert varying degrees of toxic effects on the swimming capability of Daphnia under otherwise identical test conditions. Certain concentrations result in certain percentages of Daphnia being no longer capable of swimming after the test. The concentrations resulting in zero or 100 per cent immobilisation are derived directly from the test, whereas the 24h EC 50 value is determined by calculation.

° Conditions for the validity of the test

- The mortality in the control group should not exceed 10 per cent at the end of the test.
- The oxygen concentration at the end of the test must be > 70 per cent of the air saturation value at the temperature used.

- Test Daphnia should not have been trapped at the surface of the water, at least in the control group.

- Reference runs should be made periodically to ascertain the reliability of the test system. The results with a reference substance should be within the normal range for the laboratory running the test.

- If the EC 50 is not calculable due to an inadequate number of intermediate response levels, then it is acceptable to merely report the highest concentration producing no immobility and the lowest concentration causing complete immobility, provided that the concentration factor between doses was $\leqslant 1.8$.

B. DESCRIPTION OF THE TEST PROCEDURE

° Preparations

Equipment and material

Normal laboratory apparatus and equipment should be used. Equipment which will come into contact with the test solutions should preferably be all-glass; this glassware should be cleaned with solvents known to remove previously tested chemicals.

Dilution water: any water, either reconstituted or natural water, can be used, provided that it will sustain good reproduction in Daphnia (see Conditions for the validity of the test). In addition, the dilution water should meet the criteria given in reference (3). Examples of reconstituted water are given in references (2) and (8).

° Experimental animals

Daphnia magna, or any other suitable Daphnia species, less than 24 hours old at the beginning of the test, laboratory bred, free from known diseases and with a known history (breeding method, pretreatment) are used in this test. Details of algal culturing techniques for feeding purposes and suitable Daphnia breeding techniques are given in reference (8). It is advisable to use the same species in this Phase I range-finding test as in the Phase II reproduction test.

° Performance of the test

- At least 20 animals, preferably divided into four batches of five animals each, should be used at each test concentration or control.

- Loading: at least 2 ml of test solutions should be provided for each animal.

- The test temperature should be between 18 and 22°C, but for each single test it should be constant within ± 0.5°C.

- A light-dark cycle is optional, complete darkness is allowable for the acute test.

- The concentrations are made up in a geometric series, preferably without using any substances, such as organic solvents, emulsifiers or dispersants. If such substances have to be used, they should be commonly-used adjuvants and not be toxic in themselves at the levels used. Neither should they have a synergistic or antagonistic effect on the toxicity of the substance tested. In no case should the concentration of organic solvents, emulsifiers or dispersants exceed 0.1 ml/l.

- The concentrations may be either measured or nominal, i.e. calculated, based upon the amount of material used in preparing the solution.

- The test solution should be prepared before introduction of the Daphnia.

- The test solutions should not be aerated.

- The Daphnia must not be fed.

- The highest concentration to be tested should not exceed 1 g/l.

- Concentrations sufficient to lead to zero and 100 per cent immobilisation and preferably the 24h EC 50 (see Test report, below) should be tested together with a control.

- The pH and the oxygen concentration of the control and all the test concentrations should be measured at the beginning and the end of the test; the pH of the test solutions should not be modified.

- Volatile compounds should be tested in completely filled closed containers, large enough to prevent lack of oxygen.

PHASE II - 28-DAY REPRODUCTION TEST

The results of the acute immobilisation phase of the test are to be used to determine, with judgement, the concentration levels to be used in the reproduction test proper. It is suggested that this reproduction test be carried out using a geometrical concentration series of at least five concentrations with an interval of at least $\sqrt{10}$, starting at about the 24h EC 50 and ending at 1/100 of the 24h EC 50. If necessary lower concentrations are to be tested.

1. INTRODUCTORY INFORMATION

° Prerequisites

- Water solubility
- Vapour pressure
- Chemical stability in water and light
- Results of a test on ready biodegradability (see Test Guidelines 301 A-E)
- 24h EC 50 in Daphnia

° Guidance information

- Structural formula
- Purity of the test substance
- n-Octanol/water partition coefficient
- Methods of analysis for the quantification of the test substance in water

° Qualifying statement

For chemicals with low solubility under test conditions, it may not be possible to quantitatively determine the LC 50.

° Recommendations

- Instead of a test of two weeks duration in which three batches of young should be born per female, a test of three or four weeks may be preferred in order to obtain a more thorough judgement of the

influence of the test substance on mortality and reproduction: in this period about six to nine batches of young should be born per female.

- It is recommended that a statistical test (such as an analysis of variance) be used to determine whether the test replications can be analysed together.

° Standard documents

See references (1) to (7), Section 4, Literature.

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

° Definitions and units

Static test is a test with aquatic organisms in which no flow of test solution occurs. (Solutions may remain unchanged throughout the duration of the test.)

Semi-static test is a test without flow of solution, but with occasional batchwise renewal of test solutions after prolonged periods (e.g. 24 hours).

Flow-through test is a test in which water is renewed constantly in the test chambers, the test substance being transported with the water used to renew the test medium.

LC 50 is the median lethal concentration, i.e. that concentration of the chemical in water killing 50 per cent of a test batch of the Daphnia within a particular period of exposure (which must be stated).

° Reference substances

No reference substances are recommended for the reproduction test phase. Nevertheless, if a reference substance has been tested in the acute test, the results should be given here.

- Principle of the test method

In the reproduction phase of the test, effects on the mortality and the reproductive capacity and other signs of intoxication in Daphnia are used as indications of the toxicity of a substance added to water. For this purpose, the test organisms are exposed to solutions containing the test substance in various concentrations for a period of not less than two weeks, but long enough for the development of at least three broods. The mortality, the time of the first production of young, the number of young born and the signs of intoxication observed are compared with the corresponding parameters in the controls.

- Conditions for the validity of the test

- The mortality in the controls should not exceed 20 per cent at the end of the test.
- The oxygen concentration must have been >70 per cent of the air saturation value throughout the test.
- The pH for the controls, and for at least the most concentrated solutions, must be known throughout the test; the deviation from the initial value at $t = 0$ should be ≤ 0.3 units.
- The first young should have been born in the controls after a maximum of nine days.
- The average cumulative number of young per female in the controls after three broods, should be > 20 at a temperature of 20°C $\pm$ 0.5°.
- If the recommended concentration scheme was followed and no effect on reproduction is detected, then the results may be reported as being greater than the highest concentration tested.

B. DESCRIPTION OF THE TEST PROCEDURE

° Preparations

Equipment and material

Normal laboratory apparatus and equipment should be used. Equipment which will come into contact with the test substances should preferably be all-glass; this glassware should be cleaned with solvents known to remove previously tested chemicals.

Dilution water: any water, either reconstituted or natural water, can be used, provided that it will sustain good reproduction in Daphnia (see Conditions for the validity of the test). In addition, the dilution water should meet the criteria given in reference (3). Examples of reconstituted water are given in references (2) and (8).

° Experimental animals

Daphnia magna less than 24 hours old at the beginning of the test, laboratory bred, free from known diseases and with a known history (breeding method, pretreatment) are used in this test. Other Daphnia species may be used provided that the relevant reproduction parameters are comparable to those of Daphnia magna. Details of algal culturing techniques for feeding purposes and suitable Daphnia breeding techniques are given in reference (8).

In the 28-day reproduction test food (in any quantity) of any kind that meets the criteria of reproduction given in Conditions for the validity of the test, above, is acceptable. Overloading of the test solutions with food should be avoided in order to minimise sorption of the test substance. Log-phase unicellular green algae are generally suitable.

° Performance of the test

- This reproduction test should not be carried out in a static test system: either a semi-static or flow-through system must be used. The renewal period should be guided by the chemical analysis and (if applicable) the oxygen level in the test solution; the solutions should be renewed at least once every 48 hours (if desired, on Monday, Wednesday and Friday).

- Volatile substances should be tested in completely filled closed containers, large enough to prevent the oxygen concentrations falling below 70 per cent of the saturation value. An almost-closed flow-through system may also be used.

- At least 40 animals, preferably divided into four batches of ten animals each, should be used at each test concentration.

- Loading: at least 40 ml of test solution should be provided for each animal.

- The test temperature should be between 18 and 22°C, but for each single test it should be constant, within ± 0.5°C.

- A light-dark cycle is necessary: 8 hours darkness and 16 hours light are recommended.

- The concentrations are made up in a geometric series, preferably without any substance, such as organic solvents, emulsifiers or dispersants. If such substances have to be used, they should be commonly-used additives and should not themselves be toxic at the concentrations used. They should also not interact to alter the toxicity of the substance under test. In no case should the concentration of organic solvents, emulsifiers or dispersants exceed 0.1 ml/l.

- The test solutions must be prepared before introduction of the Daphnia.

- Samples of the test substance should be taken at the beginning and during the test: the actual concentration must not drop below 80 per cent of the nominal concentration. Aeration of the test solutions is permissible, unless this would cause the actual concentration of the test substance to drop below 80 per cent of the nominal concentration.

- When more than 20 per cent of the test substance would be lost through volatility, the test should be carried out either in a flow-through system or in an enclosed container of sufficient size to ensure that the oxygen level does not fall below 70 per cent of the saturation value.

- The Daphnia should be fed at least daily.

- The oxygen concentration in all test solutions should be checked once every 48 hours (if desired, every Monday, Wednesday and Friday).

- The live and dead Daphnia of the "parental" generation (P) are counted and the dead specimens removed: this should preferably be carried out daily, but at least every two days (Monday, Wednesday and Friday).

- The presence of eggs in the brood pouch, males or winter eggs must be recorded. The condition and size of the P generation should be visually compared with the controls.

- Test substances of low toxicity should not be tested at concentrations exceeding 1 g/l.

- When the parental animals are about seven days old, the first young Daphnia emerge from the brood pouch, after which a new batch appears every two to three days. These batches are called "broods" of the F_1 (filial 1) generation.

- The newborn young of the F_1 generation should be counted at least three times a week, with an interval of 48-72 hours (e.g. Monday, Wednesday and Friday) and their visually estimated condition recorded. After counting and examination, the young are poured away. The presence of eggs from which no young have emerged on the bottom of the test vessel is checked for and recorded.

- If the renewal scheme is used, the glassware must be emptied out and food residues removed at renewal. It is recommended that the glassware be rinsed with distilled water and kept as a coded series for the following renewal. Each test unit therefore has two vessels which are used alternately. If flow-through systems are used, these should be cleaned out at intervals of at most twice a week.

- The pH of the controls and of at least the most concentrated solutions is checked before and after each renewal: if necessary the pH of the other solutions should also be checked. The results of these measurements are recorded.

- Test duration: the minimum duration of the test is 28 days, in which period not less than three broods of the F_1 generation must have appeared in the controls. If this is not the case, the test must be continued until the third brood in the control is complete. If desired, the test can be continued for a total period of three to four weeks, even if three broods are born within three weeks (see Recommendations, above).

3. DATA AND REPORTING

° Test report

For both phases of the test report:

- Test substance:
  - chemical designation
  - additional designations (e.g. trade name)
  - empirical formula
  - manufacturer
  - batch number
  - degree of purity
  - date of sampling
  - water solubility
  - vapour pressure
  - biodegradability
  - chemical stability in water and daylight
  - n-octanol/water partition coefficient
- Information about test organism: source of Daphnia, any pretreatment, breeding method (including source, kind and amount of food, feeding frequency)
- Description of the test method or reference to the method used
- Conditions of testing:
  - carriers and/or additives used and their concentrations; if it is observed that the stability or homogeneity of the test solution cannot be maintained, then care should be taken in the interpretation of the results and note made that these may not be reproducible
  - dilution water: source and chemical and physical characteristics including at least hardness, pH, Ca/Mg ratio, Na/K ratio, alkalinity

- test ... temperature
- light ... quality, intensity and periodicity
- all ... measurements of pH and oxygen level made dur...ng the test, preferably in tabular form
- rest... ...lts and date of test performed with ref...rence substance if available
- des...cription of test vessels: volume of so...tion, number of test organisms per vessel, nu...er of test vessels per concentration, co...itioning of the test vessels, the in...oduction of the test substance in the di...tion water
- in ...ase of renewal, the renewal procedure and sc...me; in case of flow-through, the test su...tance delivery system, the flow-rate, pe...odicity of cleaning and technique used
- i... ...easured, the actual concentrations of the t... substance and the dates of measurement

- Numbe... ...nd percentage of Daphnia that showed any adver... effect in the controls and in each treat...t at each observation period and a descr...ion of the nature of the effects observed (e.g. ...mobilisation, mortality) in tabular form
- Descr...on or reference to statistical procedures appli...
- Any ...r effects differentiating organisms in tests and controls

For the 24 hour EC 50 (acute immobilisation) phase also report:

- The 24h EC 50 preferably with 95 per cent confidence limits, either by computation or graphically and the method applied. For the determination a probit method should be used.
- If possible, the slope of the concentration response curve with its 95 per cent confidence limits
- The highest tested concentration producing no immobile Daphnia

- The lowest tested concentration producing 100 per cent immobile Daphnia

- Any other effect observed and the concentration at which it occurred

For the reproduction phase also report:

- The EC 50 (immobilisation) and LC 50 values as far as possible at 24 hours, 48 hours, 96 hours, 7 days, 14 days and at the end of the test, preferably with 95 per cent confidence limits, either by computation or graphically and the method applied. For the determination a probit method should be used.

- The length of time for the first brood for each concentration

- The number of young alive in each test vessel at any given day at which counts were made (the minimum requirement is for counts on Mondays, Wednesdays and Fridays)

- The number of dead young on each day of counting

- Source, kind and amount of food, feeding frequency

For each of the above a statistical analysis of the homogeneity of replicate results for each concentration should be made. If homogeneity is found, it should be determined, through an appropriate statistical analysis, whether a significant difference exists between the control and the test concentrations.

Then report:

- The highest concentration tested at which no significant difference is found versus the controls with respect to mortality, reproduction and other observed effects

- The lowest concentration tested with significant difference versus the controls

Any other parameter can be reported at the option of the study director.

4. LITERATURE

° Standard procedures

(1) International Organization for Standardization (ISO): ISO Document TC 147/SC 5/WG 2, DIS 6341, 30 August 1979

(2) DIN Testverfahren mit Wasserorganismen 38412 L1 und L15

(3) Acute and Chronic Toxicity Test Standards using Daphnids in Static, Renewal or Flow-through Systems. Toxic Substances Control Act, Section 4. Office of Testing and Evaluation, Office of Toxic Substances, U.S. Environmental Protection Agency, Washington D.C.

(4) U.S. Environmental Protection Agency 1978: Registration of Pesticides in the United States - Proposed Guidelines, Federal Register, Vol. 43, N° 132, July 10 1978

(5) AFNOR T 90301 (French standards)

(6) NEN 6501 (Dutch standard on acute test)

(7) NEN 6502 (Dutch standard on reproduction test)

° Other

(8) D.M.M. Adema, in Degradability, Ecotoxicity and Bioaccumulation: the determination of the possible effects of chemicals and wastes on the aquatic environment, Chapter 5, Government Publishing Office, The Hague, The Netherlands 1980

(9) D.M.M. Adema, Daphnia magna as a test animal in acute and chronic toxicity tests, Hydrobiologica 519, 2, 125-134 (1978)

(10) K.E. Biesinger and G.M. Christensen, Effects of various metals on survival, growth, reproduction and metabolism of Daphnia magna, J. Fish. Res. Board of Canada 29 (12), 1691-1700 (1972)

(11) R. Cabridenc, Study D.8369, Commission of the European Communities, Inter-laboratory Test Programme concerning the Study of the Ecotoxicity of a Chemical Substance with respect to the Daphnia, 1979

(12) J.H. Canton and D.M.M. Adema, Reproducibility of short-term and reproduction toxicity experiments with Daphnia magna and comparison of the sensitivity of Daphnia magna with Daphnia pulex and Daphnia cucullata in short-term experiments, Hydrobiologia 519, 2, 135-140 (1978)

(13) J. Finney, Statistical Methods in Biological Assay, Griffin Ltd., Weycombe, U.K. 1978

(14) J.T. Litchfield and F. Wilcoxon, A simplified method of evaluating dose-effect experiments, J. Pharmac., exper. Ther. 96, 99-113, (1949)

(15) C.E. Stephan, 1971, Methods for calculating an LC 50, in F.L. Mayer and J.L. Hameling, Aquatic Toxicology and Hazard Evaluation, American Society for Testing and Materials, STP 634, pp. 65-84 (1971)

PROCEDURE FOR USING PIMEPHALES PROMELAS IN AN ACUTE FLOW-THROUGH BIOASSAY

GENERAL

Fathead minnow (Pimephales promelas) has been chosen as the test species for conducting acute effluent flow-through tests. It can be easily cultured in the laboratory thus assuring a constant and readily available supply of organixms. The minnow is a native species to Indiana, important to the receiving water, sensitive to the toxicants expected and can be transported easily for on-site work. It has been used extensively in toxicity testing of complex effluents and single toxicants.

A laboratory population of Fatheads is maintained to serve as a supply for both the acute tests and as a source of eggs for embryo-larval tests. They are reared in a Frigid Units, Inc. Living Stream at 20° ± 2° C. THey are fed a diet of trout chow and Daphnia magma as recommended in "The Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish, and Blue Gills." All fish are quarantined for 2 weeks prior to their introduction into the general laboratory population. Handling, hoding and maintenance procedures generally follow those recommended in the reference mentioned above. Disease treatment during quanantine also follows the same reference. In addition, precautionary measures are taken, such as sterilization of tanks and equipment.

Fish in the general population are maintained on a 16 hour light, 8 hour dark photoperiod. Lighting is supplied via flourescent fixtures (G.E. 110 watt high output) mounted 6 feet above the water surface. Breeding fish are held in 60 liter aquariums with 2 males and 4 females per tank. Fish in the breeding chambers are subjected to a variable light cycle as mentioned in Standard Methods. THis, coupled with a gradual change to warmer water, induces breeding.

Clay tiles cut in half serve as the egg bearing substrate. Eggs are either allowed to develop in the breeding chambers or transferred to rocking egg cups for use in embry-larval tests. Eggs cups are 1½ inch PVC pipe cut into 10 cm lengths and capped on one end with Nitex screen. THey travel through a vertical distance of 4 cm, 12 times a minute.

All aquariums are sheltered from disturbance with black plastic. THis also prevents exposure to differing light cycles. At this time, reconstituted distilled water is used to replenish the sytems, however, it is hoped that a flow-through system using dechlorinated tap water by-passing the copper lines of the building can be installed.

Disease treatment during quarantine follows hat recommended in the above mentioned reference. In addition, precautionary measures such as sterilization of tanks and equipment are taken.

Transport to and from the on-site mobile bioassay unit is accomplished with water filled plastic bags placed in water filled coolers. The void space a the top of the bag is filled with oxygen. The bag is placed in the acclimation tank in the mobile unit to equalize temperature and then the fish are released into 100% holding water. This water is gradually changed to 100% dilution water at least 24 hours and 3 water volume changes before the test begins. Temperature is also adjusted to the test temperature of 21 $\pm$ 2 ^{o}C.

The Flow-through diluter system mounted in the mobile unit is an Ace Glass, Inc., solenoid actuated proportional filuter. It is prefaced by a constant temperature water bath. Operation is controlled with time delay relays. A volume of 500 ml is distributed to each of 14 exposure tanks which are duplicates of 7 effluent concentrations. Each exposure tank has a volume of 15.4 liters and a water depth of 20 cm. A flow of 6-10 tank volumes per day will be used depending upon the operation of the plant and the effluent variability.

Procedure for Using Pimephales Promelas in an Acute Flow-Through Bioassay

All construction materials involved in exposure are glass, teflon, tygon or stainless steel. The apparatus is disassembled and cleaned between tests using detergent, cid, organic solvents and distilled water. All equipment is rinsed with dilution water just before a test.

Ten fish are randomly distributed to each exposure tank, onf fish at a time. Diluter volmes and effluetn concentrations are based on a 24 hour range finding test. The operation of the diluter is checked daily during the test. Test methods will follow accepted practice for conducting bioassays with fish. Mortality will be observed and an LC_{50} reported.

TEST PROCEDURES

In general, test procedures will follow:

Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms EPA-600/4-78-012

Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians EPA-660/3-75-009

Standard Methods for the Examination of Water and Wastewater, 15th edition 1980, APHA-AWWA-WPCF

Quality Assurance Guidelines for Biological Testing EPA-600/4-78-043

Standard Test Methods for Evaluating the Acute Toxicity of Water to Fresh-water Fish, ASTM D-1345-59 (app. 1977)

TEST PROTOCOL

1 MONTH PRETEST

Make sure there will be a sufficient stock of test organisms at time of test. (200 needed per test)

Make sure population is healthy.

Check:
- Feeding schedule
- Photoperiod (16 hr. light 8 hr. dark)
- Water temperature (22 $\pm$ 2 ^{o}C)
- Percent mortality (<5%)
- Water parameters
- Dissolved oxygen (>60%)

7 DAYS PRETEST

Make sure population of test organisms is in good shape. Grade the fish so that all test fish will be within 0.5 and 5.0 grams each, of the same year class and that the longest fish will not be more than 1.5 times the length of the shortest, at the time of testing. Check the mortality record.

48 HOURS PRETEST

Terminate feeding and transport fish to on-site location. Adjust the temperature in the acclimation tank to the test temperature (22 $\pm$ 2 ^{o}C) with no more than a 2 ^{o}C change in a 24 hour period. Fish must be at the test temperature for 24 hours prior to the test. Gradually change from 100% holding to 100% dilution water while making temperature adjustment. Fish must be in 100% dilution water 24 hours and 5 water volume changes before the test. Maintain a constant record of mortality during transport and acclimation. It should not go above 5% of the total test population. Conduct a 24 hour range finding test to determine the concentrations necessary for the definitive test.

24 HOURS PRETEST

Start the operation of the diluter and make any adjustments to volumes, temperature or flow rate that are necessary. Exposure chambers should go through at least 3 water volume changes before test begins. Measure dissolved oxygen of dilution water. Aerate only the dilution water if it is necessary to bring the D.O. above 60%. Start any recording devices and calibrate to the range expected. Make sure all pumps, solenoids, valves, and timing devices are functioning properly.

1 HOUR PRETEST

Select and randomly distribute fish. Add 1 fish at a time to a randomly chosen aquarium until all exposure tanks contain 10 fish. This procedure should be accomplished as rapidly as possible.

START OF TEST

Start timing after last fish is distributed. Reset the timer and cycle counter on the diluter control panel at this time. Observe all exposure tanks and record the number of live fish in each. Notice immediate vigor and general activity of all fish. Measure and record dissolved oxygen, pH, temperature and specific conductance in each tank. Take water samples for analysis, in control, high, medium and low concentration exposure tanks. Measure temperature in at least one tank hourly or use recorder.

DURING TEST

Record the number of live organisms at 0, 1.5, 3, 6, 12, 24, 48, 72 and 96 hours. Measure dissolved oxygen and take a water sample immediately in any tank where all the fish die. Measure dissolved oxygen, pH and specific conductance every 24 hours or more. Take a water sample every 24 hours in control, high, medium, and low tanks. Measure total ammonia in any tank fish seem stressed. Note and record any sublethal effects. Note and record any unusual events or characteristics. Check operation of diluter every 24 hours. Make sure there is enough dilution water in the supply tank to last until the next observation period.

END OF TEST

After 96 hours, make any final observations and take final water samples. Check diluter operation. Record any data necessary. Remove fish and discard. Rinse diluter with dilution water. Dismantle and clean the diluter, tubing, pumps and tanks with detergent and acid and rinse with distilled water.

9-28-79

32-Day Embryo-Larval Test Procedure

Tentative Guidelines for Flow-Through Early Life Stage Toxicity Tests with Fathead Minnows

1. In an Early Life Stage Toxicity Test with fathead minnows, organisms are exposed to toxicant during part of the embryonic stage, all of the larval stage and part of the juvenile stage. The organisms are examined for statistically significant reductions in percent hatch, percent survival, and weight in order to determine upper and lower chronic values.

   A lower chronic value is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause the occurrence (which was statistically significantly different from the control at the 95% level) of any specified adverse effect, and (c) below which no tested concentration caused such an occurrence.

   An upper chronic value is the lowest tested concentration (a) in an acceptable chronic test, (b) which caused the occurrence (which was statistically significantly different from the control at the 95% level) of any specified adverse effect and (c) above which all tested concentrations caused such an occurrence.

2. Not enough information is currently available concerning early life stage tests with fathead minnows to allow precise specification of details for most aspects of the test. Enough such tests have been conducted and enough aspects have been studied, however, to indicate that these Guidelines are

appropriate. A prudent course of action for anyone planning to conduct such tests would be to initially conduct a test with no toxicant to gain experience and to determine if the requirements of items 10, 11, 19, 20, 25 and 26 are met using the planned water, food, procedures, etc. General information on such things as apparatus, dilution water, toxicant, randomization of test chambers and organisms, and methods for chemical analyses, can be found in Draft #9 of the proposed ASTM Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians.

3. Tests should be conducted with at least five toxicant concentrations in a geometric series and at least one control treatment. The concentration of toxicant in each treatment, except for high concentration and the control treatment, should usually be 50 percent of that in the next higher one. (C)

4. If a solvent other than water is used to prepare test solutions, a solvent control (at the highest solvent concentration present in any other treatment) is required in addition to the regular control, unless such a control has already been tested in the same water with the same species of fish, food, and test procedure and the water quality has not changed significantly. A concentration of solvent is acceptable only if it is (or has been) shown that that concentration or a higher one does not cause a difference (increase or decrease in any of the kinds of data specified in item #27) from control organisms that is significant at the 95% level using a two-tailed test.

5. For each treatment (toxicant concentration and control) there must be (a) at least two replicate test chambers each containing one or more embryo cups; (b) at least 100 embryos divided equally between the embryo cups; and (c) at least 30 young fish divided equally between the test chambers.

6. Two test chambers have been used routinely:

   a. Twenty fish have been tested in a chamber which is 16 cm x 44 cm x 18 cm high with a 16 cm x 18 cm 40-mesh stainless steel screen 6 cm from one end, with a water depth of 12.8 cm and with a flow rate of 190 ml/minute.

   b. Fifteen fish have been tested in a chamber which is 6.5 cm x 17.5 cm x 9.5 cm high with a 6.5 cm x 9.5 cm 40-mesh stainless steel screen 2 cm from one end, with a water depth of 4.4 cm and with a flow rate of 15 ml/minute.

   All of the above are inside dimensions. In both test chambers the water depth is controlled by a standpipe located in the smaller screened compartment with the test solution entering at the other end of the test chamber.

7. Embryo cups should be glass cylinders about 4.5 cm inside diameter and about 7 cm high with 40-mesh nylon or stainless steel screen glued to the bottom. The embryo cups must be suspended in the test chamber in such a way as to insure that the organisms are always submerged and that test solution regularly flows into and out of the cup without agitating the organisms too vigorously. A rocker arm apparatus driven by a 2 r.p.m.

motor and having a vertical-travel distance of 2.5 - 4.0 cm has been successfully used, as have self-starting siphons.

8. An acceptable dilution water for early life stage toxicity tests with fathead minnows is one in which the species will survive, grow, and reproduce satisfactorily.

9. A 16-hr light and 8-hr dark photoperiod should be provided. A 15- to 30-minute transition period at "lights on" and "lights off" may be desirable. Light intensities from 10 to 100 lumens at the water surface have been used successfully, but the intensity should be about the same for all test chambers. Light should be provided by wide-spectrum (color Rendering Index > 90) fluorescent lamps.

10. Tests should be conducted at 25°C. The temperature in each test chamber should be between 24 and 26°C at all times and must be between 20 and 28°C at all times. If the water is heated, precautions should be taken to assure that supersaturation of dissolved gases is avoided and total dissolved gases should be measured at least once during the test in the water entering the control teatment.

11. The dissolved oxygen concentration should be between 75 percent and 100 percent saturation at all times in all test chambers. At no time during the test should one test chamber have a dissolved oxygen concentration that is more than 1.1 times the dissolved oxygen concentration occurring in another tank at the same time.

12. The flow rate of test solution through the test chambers must be great enough to maintain the dissolved oxygen concentration (see items 11 and 22) and to insure that the toxicant concentrations are not decreased significantly due to uptake by test organisms and material on the sides and bottoms of the chambers.

13. A test begins when embryos in embryo cups are placed in test solution and ends 32 days later.

14. Embryos and fish should not be treated to cure or prevent disease or fungus before or during a test.

15. Embryos should be obtained from a fathead minnow stock culture maintained at 25°C and a dissolved oxygen concentration between 75% and 100% saturation with a 16-hr light and 8-hr dark photoperiod. Frozen adult brine shrimp has been successfully used as a food for adult fathead minnows. The most eggs have been obtained in a 30 cm x 60 cm x 30 cm deep chamber with a water depth of 15 cm when 15 cm x 30 cm quadrants are formed with stainless steel screen and one male, one female and one or two substrates are placed in each quadrant. Half-round spawning substrates with an inside diameter of 7.5 cm and a length of 7.5 cm have been used successfully.

16. At least three substrates with embryos on them must be soaked in dilution water for at least two hours after removal from the culture unit and they should not have been in the culture unit for more than 20 hours. For each individual substrate the embryos must be gently separated and removed and

visually examined using a disecting scope or a magnifying viewer. Empty shells and undeveloped and opaque embryos should be discarded. If less than 50 percent of the embryos from a substrate appear to be healthy and fertile, all the embryos from that substrate should be discarded. Single embryos with no fungus or partial shells attached are preferable, although embryos with some fungus or partial shells attached and clumps of two or three embryos (with or without separation) have been used successfully. Only healthy fertile embryos that are known to have been fertilized for less than 24 hours should be placed in embryo cups. An approximately equal number of healthy, fertile embryos from one substrate should be impartially distributed to each embryo cup and the process repeated for at least two more substrates until the proper number of embryos have been placed in each cup.

17. Twenty-four hours after they were placed in the embryo cups, the embryos should be visually examined under a disecting scope or magnifying viewer and all dead embryos discarded. Embryos that are alive but heavily fungused should be discarded and subtracted from the number used as the basis for the calculations of percent hatch. Each day thereafter the embryos should be similarly examined without the use of a scope or viewer.

18. In each treatment, when hatching is about 90% completed or 48 hr after first hatch in that treatment, the live young fish should be counted and an appropriate number (30 if available, otherwise all) impartially selected and transferred from the embryo cup(s) to the test chambers. If necessary,

fish can be transferred from one test chamber to another within a treatment to achieve equal numbers in the test chambers. Unhatched embryos should be left in the cups to see if they hatch. The range of time-to-hatch in each cup should be recorded.

19. A test should be terminated if the average percent hatch in any control treatment is less than 50 percent or if the percent hatch in any control embryo cup is more than 1.6 times that in another control embryo cup.

20. The flow rate, size of the test chamber and the amount of food added should be such that the average weight of the controls at the end of the test would not be significantly greater if only half as many fish were tested.

21. Each test chamber containing live fish over two days old must be fed live newly hatched brine shrimp at least two times a day at least 6 hrs apart (or three times a day about four hours apart) on days 2-5 after hatch and at least five days a week thereafter. They must be fed at least once a day on all other days. Other food may also be provided in addition to the above. The amount of food provided to each chamber may be proportional to the number and size of fish in the chamber, but each chamber must be treated in a comparable manner. Quantifying the amount of live newly hatched brine shrimp to be fed is difficult, but the fish should not be overfed or underfed too much. A large buildup of food on the bottom of the chamber is a sign of excessive overfeeding. A sign of not feeding enough of the right kind of food is that in a sideview the abdomen does not protrude.

22. Test chambers should be cleaned often enough to maintain the dissolved oxygen concentration (see items 11 and 12) and to insure that the toxicant concentrations are not decreased significantly due to sorption by matter on the bottom and sides. In most tests if the organisms are not overfed too much and the flow rate is not too low, removing debris from the bottom once or twice a week should be adequate. With some toxicants that promote growth of bacteria the sides and bottoms will have to be cleaned more often. Debris can be removed with a large pipette and rubber bulb or by siphoning into a white bucket. The pipette and bucket should be examined to insure that no live fish are discarded.

23. Temperatures should be recorded in all test chambers once at the beginning of the test and once near the middle of the test. In addition, temperature should be recorded at least hourly in one test chamber throughout the test.

The dissolved oxygen concentration should be measured in each treatment once near the beginning of the test and near the 21st and 28th days of the test.

Hardness, pH, alkalinity, and acidity should be measured once a week in the control treatment and once in the highest toxicant concentration.

The concentration of toxicant should be measured at least twice a week in each treatment.

24. Dead fish should be removed and recorded when observed. At a minimum live fish should be counted 11, 18, 25 and 32 days after the beginning of the test. The fish should not be fed for the last 24 hours prior to termination. At termination the number of fish that are visibly (without the use of a disecting scope or magnifying viewer) grossly abnormal in either swimming behavior or physical appearance should be determined. Also at termination the weight (wet, blotted dry) of each fish that was alive at the end of the test should be determined. If the fish exposed to toxicant appear to be edematous compared to control fish, determination of dry, rather than wet, weight is probably desirable.

25. A test is not acceptable if the average survival of the controls at the end of the test is less than 80 percent or if survival in any control chamber is less than 70 percent.

26. A test is not acceptable if the relative standard deviation (RSD = 100 times the standard deviation divided by the mean) of the weights of the fish that were alive at the end of the test in any control test chamber is greater than 40 percent.

27. Data to be statistically analyzed are:

    percent normal hatch

    percent survival at end of test (based on fry, not embryos)

    percent normal at end of test (based on fry, not embryos)

    weights of individual fish that were alive at end of test.

For percent data, the test chambers are treated as the replicates. For weights the individual fish are used as the replicates unless a two-tailed F test indicates that differences between replicate test chambers are not negligible.

For statistical analysis weights should not be transformed, but percent data should be transformed using the equation:

$$A = 1/2\left(\arcsin\sqrt{\frac{x}{N+1}} + \arcsin\sqrt{\frac{x+1}{N+1}}\right)$$

where N = number of organisms tested

x = number of organisms hatched, alive or normal.

(Dixon and Massey, Introduction to Statistical Analysis, 34d Ed. 1969 p. 324.)

28. Data should be analyzed using Bartletts' test and one-way analysis of variance to obtain information concerning the upper and lower chronic values. If the one-way analysis of variance results in an F ratio that is significant at the 95% level, use Dunnett's procedure (Steel and Torrie, Principles and Procedures of Statistics, 1960, p. 111) to identify treatment means that are statistically significantly different at the 95% level.

NOTE: An alternative procedure to that described above is to remove an excess of embryos about 2-20 hours old from three or more substrates and place them in embryo cups (about 30- to 50-per cup) in the control treatment for about 24 hours. Then the appropriate number of healthy, fertile embryos are impartially placed in separate embryo cups and one cup is placed in each test chamber so that each treatment receives at least 30 embryos. When the embryos hatch, all fry are placed in the test chamber. Other aspects of the test are unchanged, except that the minimum acceptable percent survival (see item 25) may have to be reduced.

In this procedure the percent survival at the end of the test takes into account the percent hatchability. In addition, starting the test with older embryos should reduce problems with fungus and reduce the need for handling of the embryos during exposure. Also, selection of the fry to be placed in the test chamber is not necessary.

References

Benoit, D. A. and R. W. Carlson, 1977, Spawning Success of Fathead Minnows on Selected Artifical Substrates. Prog. Fish-Cult. 39:67-69.

Flickinger, S. A., 1969, Determination of Sexes in the Fathead Minnow. Trans. Amer. Fish. Soc. 198:526-527.

Gast, M. H. and W. A. Brungs, 1973, A Procedure for Separating Eggs of the Fathead Minnow. Prog. Fish-Cult. 35:54.

May, R. C., 1970, Feeding Larval Marine Fishes in the Laboratory: A Review. Calif. Mar. Res. Comm., California Cooperative Oceanic Fisheries Investigations Report 14:76-83.

This tentative procedure was written by Charles Stephan with the help of many members of the staff of the Environmental Resarch Laboratory in Duluth,

E. QUALITY ASSURANCE

The product of JMM's Environmental Research Laboratory is accurate and precise water quality data. To ensure and maintain the highest quality, ERL has always had a Quality Assurance Program. This program covers sampling protocol, laboratory control control, sample handling, and other quality assure parameters. Our program is based on the "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA, March 1979. We have prepared our own procedure and QA manual which all our analysts use.

Our sample processing procedures, as outlined below, demonstrate a strong oversight role of management in the JMM-ERL QA program. Every lab report is reviewed by the analyst, the group leader and/or the QA officer, the lab director, and the Environmental Sciences Department head so there is continuous cross checking of results. The attached flow diagram demonstrates the role of management in our QA program.

Mr. Steven C. Roesch is currently JMM's quality assurance officer and he will have the sample duties for the project. He reports directly to the lab director and supervises several people concerned with the task of maintaining the analytical quality.

The following quality control procedures are used in running all sets of analyses:

(a) A minimum of three values for standard curve quantification.
(b) Duplicates run at least every tenth sample or once per sample set, whichever is more frequent.
(c) Spikes added for recovery on at least every tenth sample in a set.
(d) Additional standards run after every tenth sample to verify standard curves.
(e) A reagent and procedure blank run with every 10 samples or per set, whichever is more frequent.
(f) A field blank is run whenever possible.

The above Q-C system is employed primarily on instrumentation analyses. Where feasible, the same principals hold in the wet chemistry area. In addition to these Q-C practices, there are a number of data validation procedures which our computer system conducts after an analysis is completed which is described in a later section.

LABORATORY OPERATIONS MANUAL

Another component of JMM quality assurance is ERL's "Laboratory Operations Manual." This document covers the quality assurance practices during the analytical bench work in the areas of inorganics, organics, and microbiology. Procedures such as sample chain of command, of temperature logs, incubators and water baths, chemical standardization and preparation, instrumentation check-out procedures, instrumentation logs, microbial water quality, microbial media tests, autoclave procedures, and safety precautions are detailed in this manual.

CHAIN OF CUSTODY

JMM-ERL's sample control and chain of command procedure conform to procedures mandated in legal arbitration. This means that samples are under lock and key and a signatory of chain of custody to document the sample integrity is provided. Examples of these forms have been included.

ROUND ROBIN LABORATORY CHECKS

ERL participation in Round Robin analyses with other laboratories is another important component of our Quality Assurance Program. As part of the California State approval system for water laboratories, ERL is evaluated by EPA and the State performance check samples and purchases ERA quality control samples. Tables 1, 2, and 3 present ERL's most recent information on analytical accuracy. These tables were conducted in conjunction with the California State approval systems for water and wastewater laboratories. The EPA check was a requirement while doing research work for that agency. The other information is part of ERL's own Quality Assurance Program.

ERL's chemical section has participated in Round Robins involving selenium, trihalomethanes, TOC, and phenols.

Another element of ERL's Quality Assurance Program involves routine standardization and calibration of the analyzers. The following are examples from our laboratory manual for Q.C. procedures currently in practice for some of the analyses to be conducted on this job.

GENERAL AAS QUALITY CONTROL

In AAS, stock standard solutions are prepared from the highest purity metals, oxides or nonhydroscopic reagent grade salts using deionized distilled water. Calibration standards are then prepared by diluting the stock metal solutions at the time of analysis.

After a calibration curve composed of a minimum of a reagent blank and three standards have been prepared, subsequent calibration curves must be verified by use of at least a reagent blank and one standardat or near the maximum contaminant level (MCL). Daily checks must be $\pm$ 10 percent of the original curve.

If 20 or more samples are analyzed, the working standard curve must be verified by running an additional standard within the range of sample values each 10 samples. Checks must be $\pm$ 10 percent of the original curve.

Reagents blanks are run for each metal determined with the sample values being corrected accordingly.

At least one duplicate sample will be run every five samples, or with each set to verify precision of the method. Checks should be within the control limit established by EPA.

Spiked aliquots will be analyzed with a frequency of 5 percent of the sample load. If the recovery is not within ± 10 percent of the expected value, the sample will be analyzed by method of standard addition.

STANDARD ADDITION

Where the sample matrix is so complex that components cannot be accurately matched with standards, the method of standard addition is used. In this method, equal volumes of sample are added to a deionized distilled water blank and to three standards containing different known amounts of the test element. The absorbance of each solution is determined and then plotted on a graph (see Figure 1). Environmental Resources Associates (ERA) standards, which are independently prepared check samples commercially obtained, are run with every AAS sample.

FLAME QUALITY CONTROL

Gas flows (oxidant and acetylene) are monitored at the start and end of each run to ensure flame integrity through analysis. The Perkin Elmer flame operations manual is followed strictly.

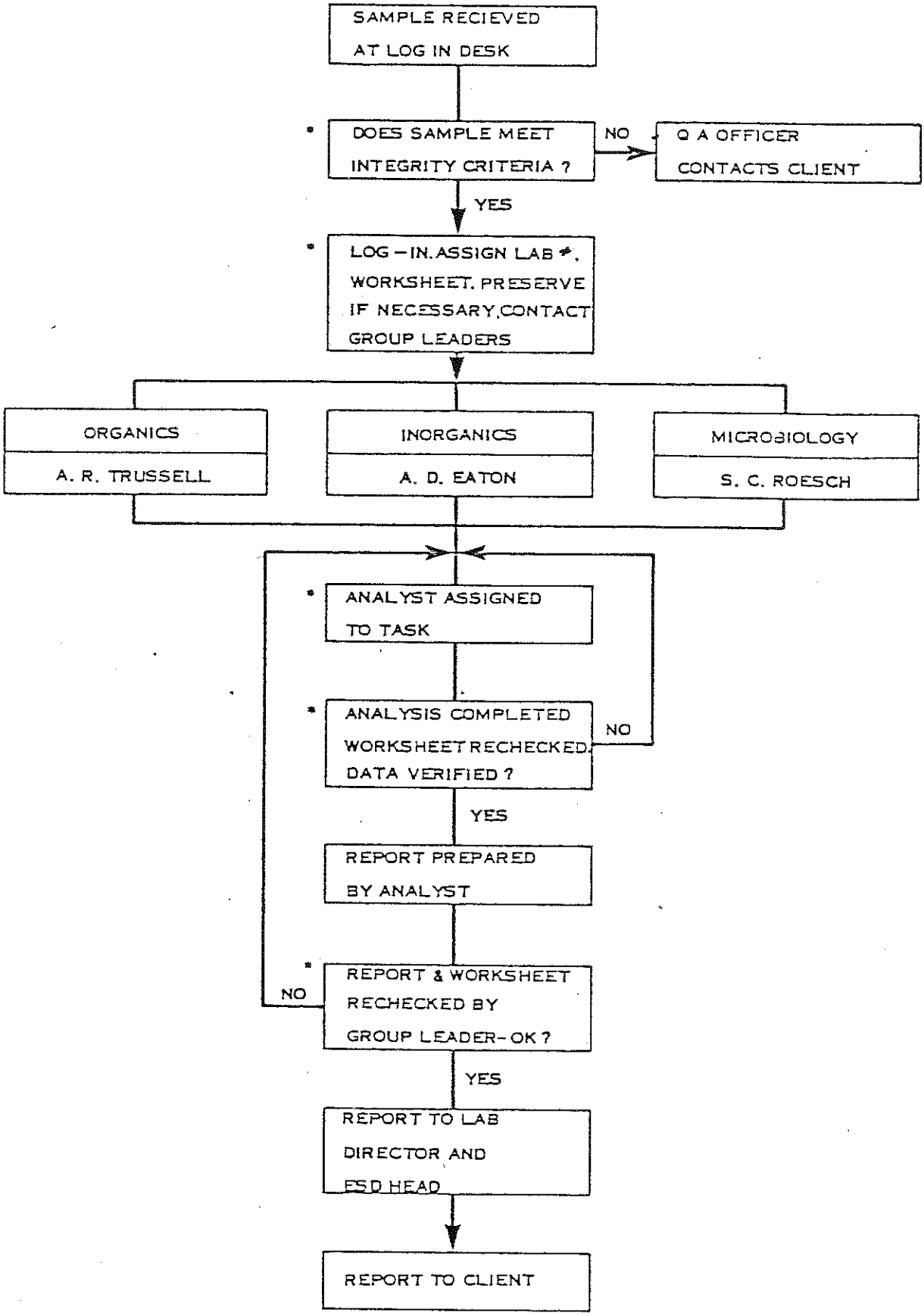
FURNACE QUALITY CONTROL

Samples are run in duplicate. If the first two injections are not within ± 10 percent of each other, more injections are made until reproducability is achieved.

ORGANICS QUALITY CONTROL

After calibrating the instrument, a standard solution is run every ten samples for TOC analyses at ERL. With the THM and VOA instruments, a single standard solution is run at the beginning of a test series.

ERL conducted an internal check for precision in TOC analysis (see Table 4). The standard deviation varied from 4-5 percent between the Sparge and Boat modes of analysis for various sample matrices.



SAMPLE PROCESSING AT JMM-ERL
KEY QA STEPS SHOWN BY *

ENVIRONMENTAL RESEARCH LABORATORY (ERL)

a Division of James M. Montgomery, Consulting Engineers, Inc.

555 East Walnut Street, Pasadena, California 91101/(213) 796-9141/(213) 681-4255 Telex: 67-5420

CHAIN OF CUSTODY RECORD

Hazardous Materials

Collector's Sample No. _____ ERL Lab No. _____

_____ _____

_____ _____

_____ _____

Location of Sampling: _____ Producer _____ Hauler ___ Disposal Site

_____ Other: _____

Client Name/No. _____ Telephone ( ) _____

Address _____

number street city state zip

Collector's Name _____ Telephone ( ) _____

signature

Date Sampled _____ Time Sampled _____ AM PM

Type of Process Producing Waste _____

Waste Type Code _____ Other _____

Field Information _____

_____

_____

Sample Allocation:

1. _____

name of organization

2. _____

name of organization

3. _____

name of organization

Chain of Possession

1. _____ _____ _____ _____

signature title inclusive dates

2. _____ _____ _____ _____

signature title inclusive dates

3. _____ _____ _____ _____

signature title inclusive dates

ENVIRONMENTAL RESEARCH LABORATORY (ERL)

a Division of James M. Montgomery, Consulting Engineers, Inc.
555 East Walnut Street, Pasadena, California 91101/(213) 796-9141/(213) 681-4255 Telex: 67-5420

ANALYSIS REQUEST

PRIORITY _____

(Explain) _____

_____

_____

PART I: FIELD SECTION

Collector _____ Date Sampled _____ Time _____ AM PM

Location of Sampling _____
name of company, disposal site, etc.

Address _____
number street city state zip

Telephone ( ) _____ Company Contact _____

ERL No. (Lab only)	Collector's Sample No.	Type of Sample*	Field Information**

Analysis Requested _____

_____

_____

Special Handling and/or Storage _____

_____

_____

PART II: LABORATORY SECTION

Received by _____ Title _____ Date _____

Sample Allocation: _____IL ____ML ____OL Date _____

Analysis Required _____

_____

_____

*Indicate whether sample is sludge, soil, etc.; **Use back of page for additional information.

TABLE 5

EPA REFERENCE SAMPLE
(4th Quarter, 1979)

LABORATORY: CA006

Parameter	Sample Number	Reported Value	True Value	Acceptance Limits	Performance Evaluation
ALL VALUES IN MICROGRAMS PER LITER (EXCEPT AS NOTED)					
Arsenic	1	23	24.1	9.13-37.4	Acceptable
	2	26	36.3	10.7-40.2	Acceptable
Barium	1	164	184	76.4-287.	Acceptable
	2	218	264	131.-390.	Acceptable
Cadmium	1	4.2	4.4	1.13-7.64	Acceptable
	2	3.2	3.3	.417-6.27	Acceptable
Chromium	1	18	21.8	9.81-33.7	Acceptable
	2	36	36.1	16.6-54.0	Acceptable
Lead	1	29	30.7	12.1-52.8	Acceptable
	2	18	17.5	3.45-36.2	Acceptable
Mercury	1	1.6	1.9	.344-3.11	Acceptable
	2	1.8	2.0	.375-3.26	Acceptable
Selenium	1	3.8	7.0	1.30-17.4	Acceptable
	2	5.1	8.4	2.17-14.3	Acceptable
Silver	1	30	30.7	6.85-56.0	Acceptable
	2	36	36.4	9.31-63.5	Acceptable
Nitrate as N (milligrams per liter)	1	5.2	6.16	3.39-8.60	Acceptable
	2	0.63	0.71	.504-.941	Acceptable
Fluoride (milligrams per liter)	1	0.35	0.40	.267-.543	Acceptable
	2	1.7	1.67	1.26-2.07	Acceptable
Chloroform	1	31	26.,1	7.19-44.0	Acceptable
	2	67	55.9	15.9-90.9	Acceptable
Bromoform	1	22	20.5	2.84-36.4	Acceptable
	2	122	102	8.98-182.	Acceptable
Bromodichloromethane	1	74	70.8	23.9-104.	Acceptable
	2	26	23.6	7.35-35.5	Acceptable
Dibromochloromethane	1	113	113	25.7-191.	Acceptable
	2	8	5.6	0.-20.1	Acceptable

TABLE 6

CALIFORNIA STATE
REFERENCE SAMPLE SUMMARY, 1979

Laboratory #151

Sample A — micrograms per liter, ug/l

	*N	True Value	Mean	Reported Value	Standard Deviation	1	2	3
Arsenic	154	25	23.0	22	8.2	0.1	4	22
Cadmium	155	7	7.8	7.1	2.1	0.3	28	0
Chromium	178	35	35.1	35	8.7	0	0	0
Iron	194	250	259	245	37	0.4	33	14
Lead	165	40	44.7	42	9.0	0.3	17	22
Manganese	164	150	149	151	17	0.1	24	21
Mercury	124	1	1.2	0.8	0.5	0.8	49	32
Selenium	116	6	7.1	5.6	3.3	0.5	8	0
Zinc	169	60	69.0	71	11.4	0.2	23	55

Sample B — milligrams per liter, mg/l

	*N	True Value	Mean	Reported Value	Standard Deviation	1	2	3
Fluoride	199	0.7	0.74	0.9	0.12	1.3	77	77
Nitrate	217	28.0	28.0	25.5	2.7	0.9	62	62
Phosphate	192	8.0	8.3	7.9	0.6	0.7	52	10

* Number of laboratories used in the calculations
Number of Standard Deviations from the Mean
Percent of laboratories closer to the Mean
Percent of laboratories closer to the True Value

TABLE 7

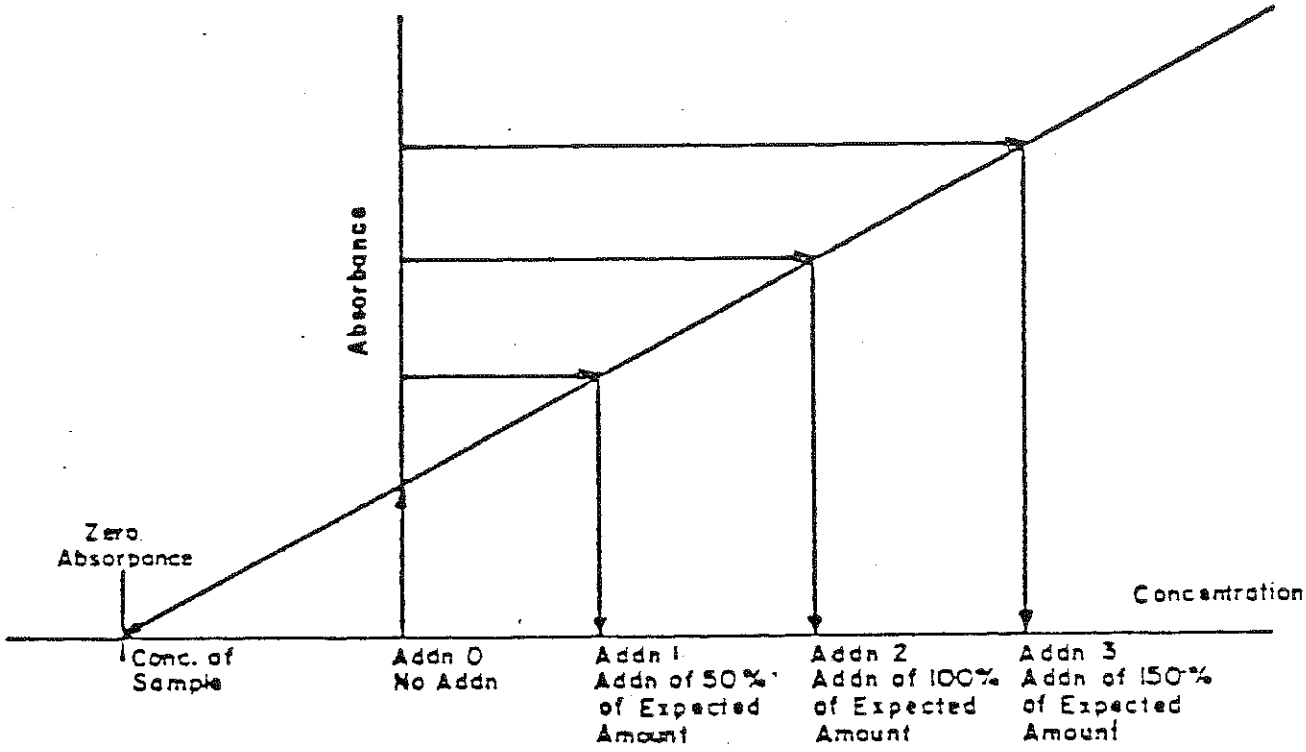
GENERAL MINERALS

June, 1979
No. 1632

	Reported	Actual
Phenol (mg/l)	0.320	0.360
pH (units)	9.1	9.0
EC (umho/cm)	1930	1950
Hardness (mg/l)	143.1	140
Calcium (mg/l)	28.8	29.0

HEAVY METALS

	No. 1632		September, 1979 No. 5739	
	JMM (mg/l)	ERA (mg/l)	JMM (mg/l)	ERA (mg/l)
Al	—	—	0.319	0.330
As	0.027	0.028	0.059	0.056
Ba	0.570-	0.550	1.04	.10
Be	0.170	0.170	—	—
Cd	0.054	0.056	0.159	0.170
Co	0.150	0.130	0.150	0.160
Cr	0.518	0.500	0.229	0.220
Cu	0.420	0.420	0.110	0.110
Fe	0.435	0.440	0.550	0.550
Hg	—	—	0.0012	0.0011
Mn	0.282	0.280	0.285	0.280
Ni	0.180	0.220	0.320	0.330
Pb	0.171	0.160	0.218	0.220
Sb	0.019	0.022	—	—
Se	0.053	0.055	0.043	0.044
V	0.123	0.110	—	—
Zn	0.351	0.330	0.288	0.280



STANDARD ADDITION PLOT

FIGURE 1

TABLE 8

TOC PRECISION ANALYSIS—0.4-1500 PPM

	Average % Difference + S.D.	Matrix
Sparge Mode	4.71 ± 6.15	Clean water
Boat Mode	3.98 ± 3.8	Industrial Waste
Boat Mode	5.83 ± 2	Sediment

TOC PRECISION FOR BOAT MODE

Sample	Average ppm	S.D.	Matrix
1	1416	88.3	Industrial Waste
2	30.9	2.6	Industrial Waste
3	232	9.2	Industrial Waste
4	117	7.3	Industrial Waste
5	42	4.6	Sediment
6	55.5	4.0	Sediment
7	376	27.9	Sludge

Quality Assurance Summary, EMS Laboratories

The quality assurance protocol for the instrumental methods of analysis (metals, nitrogen, cyanide, phenol, etc.) is best demonstrated using the attached lab bench sheets. The placement of QA samples is prescribed and recoveries of standards must be 100 $\pm$ 10%. Duplicate analysis must agree according to the following formula:

$$\frac{2\,(A - B)}{A+B} \leq .15,$$

Where A and B are the results of duplicate analyses performed on the same samples.

A confirmation standard is analyzed at the end of each set of analyses; recovery must be 100 $\pm$ 10%

Should any of the above acceptance criteria not be met all analyses which were performed after the last acceptable QA sample and before the unacceptable QA sample must be repeated.

For non-instrumental methods, standards and replicate analyses are run in parallel with the sample load; 5 to 10% of all analyses are standards; 5 to 10% are duplicates. The same acceptance criteria are used for standards and duplicates on non-instrumental methods as for the instrumental methods, except BOD.

A 200 mg/l BOD standard must be 200 $\pm$ 40 mg/l ($\pm$ 20%).

Blanks are also run with all analyses and the blank reading must not generally be above the reported detection limit.

Automated Method _____ Analyst_____ Date_____

SAMPLE CUP NO.	SAMPLE NO.	DILUTION	PEAK HEIGHT	CONCENTRATION
1	0.10 Std			
2	0.30 Std			
3	0.5 Std			
4	Blank			
5	1.0 Std			
6	Blank			
7	Sample			
8	Blank			
9	Duplicate Sample 7			
10	Blank			
11	Sample			
12	Blank			
13	Sample			
14	Blank			
15	Sample			
16	Blank			
17	Spiked Sample #7 with 0.30 mg phenol/l			
18	Blank			
19	EPA or ERA Std.			
20	Blank			
21	Sample			
22	Blank			
23	Sample			
24	Blank			
25	Sample			
26	Blank			
27	Sample			
28	Blank			
29	1.0 Std			
30	Blank			
31	Sample			
32	Blank			
33	Sample			
34	Blank			
35	Sample			
36	Blank			
37	Sample			
38	Blank			
39	Sample			
40	.50			

OBSERVATION 1. EPA, ERA or other unknown reference standards are to be used in "sample" locations.

2. The last sample or standard analyzed must be a calibration standard.

DATE:

ANALYST:

METALS

	Cd	Ni	Cu	Cr	Zn	Pb	Fe						
AMPLE #													
STANDARD													
RUE VALUE													
% RECOVERY													
A													
QA													
QA													

AMPLE #	SAMPLE #	SAMPLE #	SAMPLE #	SAMPLE #
ROSS WT.	GROSS WT.	GROSS WT.	GROSS WT.	GROSS WT.
ARE WT.	TARE WT.	TARE WT.	TARE WT.	TARE WT.
T SAMPLE	WT. SAMPLE	WT. SAMPLE	WT. SAMPLE	WT. SAMPLE